

THE ABSORPTION OF RADIO-ACTIVE COBALT AND WATER FROM
THE CECUM OF CHICKENS

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A THESIS

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology and Pharmacology

1952

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ACKNOWLEDGEMENT

The author wishes to express his deep sense of gratitude and appreciation to Dr. L. F. Wolterink of the Department of Physiology and Pharmacology for his advice and guidance throughout the course of these investigations and during the preparation of this manuscript. He wishes to thank Prof. B. V. Alfredson, Head of the Department of Physiology and Pharmacology for all the facilities given in the conduct of this research. Many thanks are due to Mr. J. Monroe for looking after the birds in the course of these experiments.

The author is indebted to the Michigan State College for the award of scholarship and to the Government of Madras, India, for granting the study leave. He wishes to express his sincere appreciation to his wife Sarojini Devi, for the help given in the preparation of the manuscript.

Finally, the author wishes to express his thanks to the U. S. Atomic Energy Commission for providing funds for the purchase of supplies and the radioactive cobalt used in this experimental work.

Introduction*

The physiological significance of the paired intestinal ceca in birds is still not clearly known. Most investigators are of the view that cecae are not essential, because cecectomy does not have any deleterious effect on the chickens nor does it lower the egg production or growth. It has been observed that cecal ablation in turkeys does not lower the digestibility of rations. Since cecal contents resemble feces, it has been concluded that cecae are essentially vestigial organs.

On the other hand ceca apparently perform some active function in digestion and absorption; therefore they may be of considerable importance in the Avian economy to conserve nutrients and water. The digestion of starches and proteins in the cecum has been reported but fat digestion has not been found. The absorptive function of cecum however, has not been clearly elucidated. Though the absorption of non-protein nitrogenous substances (presumably amides) has been shown, the absorption of vitamins and minerals is not clear. Water absorption has been considered probable by many investigators.

*The salient features of the experiment have been reported to the section of Sanitary and Medical Science at the 56th Annual Meeting of the Michigan Academy of Science, Arts and Letters, Ann Arbor, Michigan.

The microscopic anatomy reveals the presence of villi and tubular glands, suggesting that absorption can occur. It has been suggested that absorption of nutrients takes place at the narrow end and absorption of water at the dilated end of cecum.

The present investigation was undertaken to evaluate the cecal function in the absorption of water and cobalt. This report will deal specifically with:

1. Water absorption; how much and how fast is it absorbed?
2. Cobalt absorption, as indicated by Cobalt 60.
3. The relative rates of absorption of both; whether the rates are constant and what factors cause them to vary?
4. Cobalt binding; is all the cobalt available for absorption?
5. Water binding; can all the water be absorbed?

The data presented herein will also be used to illustrate the types of techniques which can be most profitably employed in further investigation. It is hoped that the procedures evolved will be generally helpful, especially in the study of binding phenomena with special reference to drug actions.

REVIEW OF LITERATURE

Ceca are not essential to chickens

Browne, T. G. (1922) observed that since digestion in chickens occurs primarily in the first part of the duodenum, if the ceca aid in digestion, their openings should have been in the anterior region where active digestion took place. He therefore was of the opinion that a majority of the digestive function is already completed by the time materials arrive at the ceca. The fact that only a small portion of the intestinal contents enter the cecae and that the contents resemble feces, led to the conclusion that ceca probably do not play any important part in digestion or assimilation.

Looper, et. al. (1929) in their study of fourteen chickens ranging in age from six days to three years observed that the developing cecae resemble intestines. They are devoid of contents until the nineteenth day of incubation, later becoming engorged with materials (as does the colon), thereby showing an early "defecatory" function. So, observing that the materials in ceca were in the nature of feces, they concluded that the ceca are not primarily concerned with digestive function but are only vestigial appendages.

Blount, W. P. (1937) repeated the opinion that ceca are dispensable and that no harm results if they are removed.

Radeff, T. (1928) observed that cecectomy in chickens has no deleterious effect on the well being of birds.

Schlotthuer, et. al. (1934) ligated the ceca of twelve turkey hens and two gobblers in an attempt to protect against enterohepatitis and observed that all of them remained normal and healthy. They also reported that the egg production and fertility are not impaired.

Mayhew, R. L. (1934) performed cecectomy on the right abdominal side and concluded that it does not interfere with growth or egg production. The cecectomized birds laid a normal number of eggs although there was a temporary cessation of egg laying probably due to temporary starvation. It was also concluded from the results of this experiment that the bad effects observed in cecal coccidiosis are not due to interference with cecal function.

Hunter, et. al. (1930) ablated the ceca of turkeys and observed that such a procedure does not lower the digestibility of the rations given to the birds.

Intact ceca may perform useful functions

Johanson, W. T. (1920) maintained that unabsorbed materials, as they pass down the intestines, enter the ceca where they are further digested and absorbed. He also observed that the contents of cecae, as they are voided into rectum and thence out the cloaca, are dark in color and of semi-solid consistency.

Digestion experiments in the lower bowel are difficult in birds because of the mixing of the urine and feces in the cloacal region. Hart, et. al. (1942) were able to exteriorize the ureters of chickens and collect urine from urodeum in their specific study of the role of the cloaca in water metabolism.

It was Maumus, J. (1901) who made a direct study of the digestive secretions in the cecum by making a cecal fistula on the right abdominal side. He chose this site because it is easy to get at the cecum. He found that the cecal juice contains a hydrolysing ferment which acts on starches and a proteolytic ferment which acts on proteins. He described the proteolytic ferment to be similar to trypsin. No digestive action on fats appears to occur in the ceca. He remarked that chickens which are fed on a carnivorous diet cannot digest starch but are able to digest protein materials. Interestingly enough, he observed that the carnivorous birds have rudimentary cecae while granivorous birds have well developed ceca. Protein digestion in ceca is suggested.

Starch digestion is also reported to take place in ceca. Radeff, T. (1928) claimed that crude fibre digestion is entirely the function of cecae in chickens. He performed quantitative experiments in two groups of chickens; one

group composed of normal birds, and another group made up of cecectomized chickens. It was found that normal chickens are unable to digest the crude fibre of barley but can digest the crude fibre of wheat to about five per cent, of oats to about seven per cent and of maize to about seventeen per cent. In cecectomized chickens the crude fibre of wheat is digestible to less than two per cent and the crude fibre of the remaining feeds is not digested at all. The normal chickens gave digestion coefficients of about seventeen per cent while cecectomized birds gave coefficients of zero.

Roseler, M. (1929) confirmed the observation that crude fibre is split in the ceca and that the crude fibre content of cecal feces is always lower than intestinal feces. Therefore the ceca are considered necessary for the digestion of cellulose.

That crude fibre digestion is not entirely due to enzymes secreted by the bird but appears to be due primarily to bacterial action, has been suggested by the following investigators.

Mangold, E. (1928) expressed the opinion that the crude fibre digestion in the ceca of chickens is due to bacterial decomposition.

Blount, W. P. (1937) also remarked that crude fibre digestion in chickens is due to the action of bacteria which convert fibre into glucose and organic acids.

That these organic acids are primarily responsible for the acid condition of the cecae appears to be a justifiable assumption. As early as 1919 Kaupp, B. F. reported the reaction in ceca to be faintly acid or neutral. Olson, et. al. (1935) also observed that the pH of the ceca is lower than that of other portions of the intestines. Similarly Farner, D. S. (1942) studying the hydrogen ion concentration of the various portions of the digestive tract in chickens, pigeons, ducks, pheasants and turkeys, found that all species showed a gradual increase in pH from the duodenum to the ileum and a decrease of pH in the ceca and colon. It has been inferred that these acids might be helpful in the digestion of crude fibre in granivorous birds. Bacterial digestion of proteins in the ceca of chickens has also been suggested.

Johanson, et. al. (1948) report that ceca have a greater number of micro-organisms than any other portion of the intestinal tract in chickens. He also reports that there are greater numbers of coliform bacteria in the cecae of birds fed on grains. It is perhaps by a double action of bacteria and enzymes that resistant materials such as crude fibre and certain proteins are digested in the ceca of chickens.

From the lack of effect of cecectomy on the nutritional status of chickens, it is apparent that, much as the human can survive very well after ablation of fairly large sections of his digestive tract, so the absolute requirement of the bird for its cecae is negligible, at least on normal rations. On minimal or borderline rations, however, it is possible that the cecae may assume greater importance. The role of the cecal bacteria is of interest in two respects. First, they may be in serious competition with their host for the essential nutrients in shortest supply. Secondly, they may actually synthesize and make available to their host certain essential micro-nutrients. In either case, evaluation of cecal function itself becomes important.

Absorptive function

The digestive function in cecae is apparently followed by an unknown amount of absorption. Browne, T. G. (1922) expressed the opinion that cecae represent miniature intestines which play an accessory role in absorption, since their microscopic anatomy is suitably adapted for the absorption of nutrients at the narrow portion of the cecum and of fluids at the dilated end (which is similar to the mammalian large intestine). Large quantities of fluid appear to pass through the cecae and but for them, most of whatever nutrients are present should have been eliminated

in the feces. Browne assumes therefore that the ceca may serve an important function in completing the absorption of nutrients.

Mangold, E. (1928), observing a higher percentage of nitrogen in cecal feces than in intestinal feces, concluded that only non-protein nitrogenous substances ("amides") can be absorbed in the cecum.

Roseler, M. (1929) always found less crude protein but a greater amount of pure protein in ceca than in other fecal materials, and therefore concluded that amides were absorbed to a high degree in the cecae of chickens.

Blount, W. P. (1927), Mangold, E. (1928) and Radeff, T. (1928) also reported cellulose digestion and subsequent glucose absorption.

Johanson, et. al. (1948) observed the synthesis of 'B' vitamins by coliform bacteria in the ceca of chickens, but the absorption of vitamins is still doubtful.

Mineral absorption is perhaps possible from the postulation of M'Gowan, J. P. (1930) who stated that the absorption and excretion of ionic iron is a reversible process occurring at the same site, i.e., the ceca of chickens. But conclusive proof is lacking.

Water absorption appears to be possible from the various evidence noted by several investigators.

Mangold, E. (1928) considered cecae to be mainly water resorptive organs from the fact that cecectomized chickens pass more moist feces.

Radeff, T. (1928) in his experiments on cellulose digestion in chickens observed that cecectomized chickens pass more moist feces than normal birds. He states that moist feces is the only clinical effect observed following cecal ablation.

Keith, et. al. (1927) determined the percentage of dry matter and percentage of moisture in the various segments of the digestive tracts of chickens at different intervals of time after feeding them with a standard meal. The percentage of moisture varied in different segments; the percentage of moisture in cecae was observed to be more than seventy per cent of the total contents with an average of less than seventy-seven per cent, but in the large intestines it was seventy-five per cent to eighty-five per cent with an average of more than seventy-eight per cent. Since the moisture content of feces in the cecum was slightly lower than that in the large intestines, it was concluded that the cecae absorb water.

Browne, T. G. (1922) in his experiments, ligatured the ceca of chickens and observed that such birds suffered diarrhoea. This was suspected to be a toxic syndrome due to blocking of circulation in the process of ligature.

Radeff, T. (1928) also observed that cecectomized chickens pass more moist feces and likewise inferred that water is not absorbed fully in the absence of ceca .

A number of investigators have presented evidence to indicate that fluids readily enter the ceca . McLeod, W. M. (1939) observed the lumen diameter at the origin of the ceca to be so small that he wondered if any intestinal contents ever entered the ceca ; although he reports that fluids enter the ceca easily.

Olson, et. al. (1935) fed chickens with equal parts of cracked corn and wheat and ten per cent dried buttermilk after fasting the birds for eighteen to twenty hours. The food was mixed with lamp black, hydro kollogg (colloidal C), carmine, Rose bengal and Trypan blue. The food with dyes was fed for twenty-four to forty-eight hours and the same foods without dyes were fed subsequently. These chickens were killed after periods ranging from a few hours to fourteen days. Samples of gastrointestinal contents were obtained from crop, proventriculus gizzard, duodenum, jejunum, illeum, right and left cecum and rectum.

It was observed that lamp black fed with food does not appear in the cecum even after two weeks of feeding while the water soluble dyes appear in the cecae in less than three hours. Colloidal C appears in the cecae in twenty-four

hours after feeding. Therefore the conclusion was drawn that only fluids entered the ceca .

Browne, T. G. (1922) observed that the materials which entered ceca resembled an overflow of the intestinal contents; only the fluids however being allowed to enter the ceca . Experiments were carried out by drenching chickens with colored fluids. The colored fluid material given orally, reached the cecae in four hours. Fluids injected into cloaca reached the blind extremities of cecae rapidly and the fluids did not appear to go beyond the cecal openings in the intestines. Browne therefore thought that the cecal tubes in chickens serve to conserve fluids by acting as reservoirs as well as functioning as organs of absorption for fluids which would otherwise have escaped with the feces.

It will be noticed that all of the evidence for cecal absorption is inferential in nature. In no case is it clear that absorption actually takes place into the bird and not into a growing population of bacteria. Since the exact arterial supply and the venous drainage from the ceca and its relation to the circulation of the lower intestines is not known, it is entirely possible that circulatory obstruction should result both from cecectomy and cecal ligation. In that case more fluid feces might well result. A more direct approach is obviously indicated.

Movements of the Ceca.

The mechanisms regulating cecal filling and emptying in chickens are not definitely known.

Browne, T. G. (1922) thought that there was some intrinsic mechanism whereby the cecal tubes were themselves responsible for forms of peristalsis causing the ceca to fill up and later to empty. The bulk of the contents, when they reach a certain maximum, appear to act as a distension stimulus which results in evacuation. If stimulated with the prick of a pin, the cecum was observed to contract towards the blind end followed by a reverse wave of peristalsis towards intestines. Browne opined that the filling of the cecum was due to suction and Valtz (1929) later expressed the same opinion.

The wriggling movement of the ceca may easily be seen immediately after death. In the live bird after canulation and in the cecal fistula operation, when the cecum is flushed with water, the contents are ejected forcibly. This indicates that the cecal contractions are fairly strong.

Mangold, E. (1928) observed that the ceca of birds discharged their contents independently of each other and also from the rest of the intestines. Autopsy revealed different contents in the cecae both quantitatively and qualitatively; the contents being viscous, foul smelling

and easily recognizable. He was of the opinion that the emptying was brought about by contraction and filling by suction due to negative pressure created by active dilatation. Since the ceca are typical viscera hanging pendulous in the peritoneal cavity, this method of filling scarcely seems possible.

Roseler, M. (1929) observed that the two ceca empty one at a time every twenty-four hours. Only one cecal emptying occurs to every seven to eleven intestinal emptyings, the exact ratio varying with the feed. Complete replacement of the cecal contents requires about five days. The cecal feces are easily distinguishable from intestinal feces.

The slow turnover of the cecal contents is in marked contrast to the rapid passage of food through the remainder of the digestive tract. Kaupp, B. F. and Ivey, J. E. (1923) showed that even in broody hens, complete passage of food through the digestive tract requires only about twelve hours on the average.

Johnson, W. T. (1920) remarked that the emptying of the ceca is a slow process due in part to their anatomical arrangement in the abdomen. Apparently some diseases establish themselves in the ceca because of their isolation from the rest of the digestive tract.

Hoelzel, F. (1930) did experiments on the rate of passage of various inert materials through alimentary canal of rabbit, guinea pig, dog, cat, rat, monkey, pigeon, hen and man using such test materials as rubber, cotton knots, glass beads, aluminum, steel, gold and silver. He observed that the rate of passage was inversely proportional to the specific gravity of materials; heavier materials passing down more slowly than lighter ones. The pigeon and chicken retain most of the heavy metals in the gizzard and in the chicken, in the duodenum and illeum as well. No test materials were found in the cecae of chickens, although food residues were present.

The local nervous mechanism regulating cecal activity is not known; but some reflexes have been observed in birds.

Reed, et. al. (1925) reported "reflexes" associated with feeding and defecation in young wrens. After swallowing food, the fledgelings developed a complicated reaction which not only led to defecation but placed the young bird in such a position that the parent bird could easily collect the excreta as voided, and remove it from the nest.

Grobbeis, Franz. (1927) observed in sparrows that all such reactions ceased when the birds became mature.

An authoritative review on cobalt metabolism in animals has been made by Marston, et. al. 1952.

Cecal Morphology

Browne, T. G. (1922) found the ceca to be about six inches long and attached to the intestines by a mesentery containing blood vessels and nerves. They open on the floor of intestines about five inches from and in front of the cloaca. He thought that ceca are perhaps a compensation for the relative shortness of the intestines in birds. Curiously enough, it is reported that hawks, pigeons and parrots have no ceca.

Hewitt, E. A. (1940) noted that the intestine was shortest in birds of prey. In chickens, pigeons, ducks and geese, the intestines appear to be four or five times as long as the entire body and in the ostrich, nine times the length of the body.

He described the intestines as composed of four divisions: (1) duodenum (2) illeum (3) cecum (4) rectum. The ceca originate at the junction of illeum and rectum and extend towards the liver, then being folded back toward the cloaca. The cecae are fifteen to twenty centimeters long; the blind extremities being larger than the proximal parts of ceca.

As noted earlier, McLeod, W. M. (1939) remarked on the very narrow lumen of the cecum at its origin, and wondered if any intestinal contents ever got in. He reported that

the wall was thicker at the narrow end and thinner at the blind end of cecum; beyond the neck the lumen diameter was about half an inch wide.

The cecal tonsil is a noteworthy feature and is of diagnostic significance to get at cecum in laparotomy of chickens.

Although the ceca are usually paired, variations are known to occur. Olson, et. al. (1935) reported the following variations: (1) well developed cecae in granivorous birds (2) rudimentary cecae in carnivorous birds (3) only one cecum (4) no cecum at all. It has been observed that the paired cecae occasionally have only a single outlet to the intestines. Normally the ceca open separately. They also report that the proximal portion of the ceca is constricted with lymphoid tissue situated in or under the mucosa near its connection to the intestine.

Microscopic Anatomy

Calhoun, M. Lois (1933) described the histology of cecum. The following structures appear in sequence as one passes from the lumen outward:

1. Lining epithelium Columnar, containing goblet cells; the whole structure similar to the mucosa of the small intestines.

2. Villi.- According to the distribution of villi, the cecum may be divided into three portions:
(a) neck with many villi (b) middle portion - a few villi (c) the vesicular blind end with no villi.
3. Tunica propria. -Above muscularis mucosa.
4. Muscularis mucosa. - Absent in many places
5. Submucosa. - White, fibrous and yellow elastic tissue containing nerves, blood vessels and lymphoid plexus.
6. Circular layer of lamina muscularis. This is displaced or replaced by lymphatic tissue in the blind end with reticular connective tissue fibres extending into circular muscle fibres.
7. External layer of longitudinal lamina muscularis.
8. Serosa. Rich in nervous elements.

The mucosa of distal two-thirds is reported to undergo degeneration as the chicken becomes older. The regression involves atrophy of the epithelium and glands and the appearance of lymphoid tissue which in turn is replaced by sclerotic fibrous tissue in the blind end of ceca .

Sisson and Grossman (1947) also report that ceca are lined with columnar epithelium containing goblet cells and that the surface is covered by villi with tubular glands emptying between them. Lymphatic tissue is reported to be abundant in cecum.

Hewitt, E. A. (1940) reported the presence of villi and glands of Lieberkuhn in the ceca of chickens.

Berry, R. J. A. (1900) stated that ceca are a special seat of lymphoid tissue which is aggregated in the cat and pigeon but is diffuse in the chicken, pig and sheep.

Looper, et. al. (1929) also observed that the proximal third of the cecum resembles the intestines and that the distal two-thirds undergoes regression with the replacement of lymphoid tissue by fibrous tissue. Lymph nodes are reported to appear in the embryonic cecae after one week of incubation.

The histological appearance of the ceca is very suggestive of absorptive function. The function of the lymphoid tissue appears to be varied.

Fulton, T. F. (1949) described lymphoid tissue to be composed of masses of cells, primarily lymphocytes held together in a frame work of reticular cells. The primary function appears to be to filter bacteria. Other functions which have been suggested from purely histologic evidence are:

1. Metabolism and transport of fat and proteins.
2. Storage of vitamins.
3. Production of hormones and antihormones.
4. Destruction of red blood corpuscles.
5. Production of lymphocytes containing antibodies.

EXPERIMENTAL PROCEDURE

Three different techniques were employed to study the function of the cecum directly. Forty four healthy chickens of varying ages, sexes and weights were utilized in these experiments. Feed and water were adequately provided and the birds housed comfortably in individual cages.

Anaesthetisation: Anaesthetisation of chickens was the delicate part of these experiments in all the three techniques. A commercial solution* of sodium pentobarbitol containing one grain (approximately 65 mgm.) of the barbiturate per ml. was the intravenous anaesthetic used. The safe doses for the anaesthetisation of different sizes of chickens were determined by trial and error. A dose of 0.1 ml. to 0.3 ml. (containing 6.5 to 19.5 mg. of sodium pentobarbitol) was safe for chickens weighing less than five hundred grams and 0.3 ml. to 1 ml. was safe and successful in heavier chickens. The intravenous injection of the anesthetic agent was given slowly and steadily into the wing vein until surgical anesthesia supervened. If the injections were made rapidly, the chickens died instantaneously probably from respiratory centre paralysis (Sollman, 1948).

*Halatol, a solution of sodium pentobarbitol in a solvent of alcohol, propylene glycol, and water. Prepared by the Jensen-Salsbury Laboratories, Inc., Kansas City, Missouri.

Laparotomy: The operation site was usually chosen on the left abdominal side of pullets and cockerels but in the mature laying hens, a corresponding site was chosen on the right abdominal side; because the enlarged left oviduct always obscured the field of operations on the left abdominal side. Although the chicken has two ovaries and two oviducts, it is usually the left ovary and oviduct which remain and develop. This was perhaps the reason that laparotomy was performed on the right abdominal side by other workers such as Maumas, J. (1901) and Mayhew, R. L. (1934).

Blood samples: In each of the three techniques, one milliliter of blood was obtained directly from the heart. The site of the needle puncture was a point, equidistant from (1) the point of the wing (2) the point of the hip and (3) the point of the keel. Another landmark of help was the fascia line above which, the puncture was always made. If the needle entered below the fascia line it entered cartilage which blocked the lumen of the needle. Blood was obtained from either side of the chest after locating the same surgical site.

Cobalt 60: The radioactivity of the Co 60 used in each of the three techniques was approximately 0.5 micro-curie per milliliter of aqueous saline solution. The cobalt was in the form of high specific activity cobaltous sulfate at a

pH of 7.5. At this pH, a portion of the cobaltous ion comes out of true solution as a colloidal hydroxide. Wolterink, Lee and Groschke (unpublished observations) have observed however, that half of the cobalt in such a solution is absorbed from the intestine of chicks in twenty minutes. The exact dose of the tracer used in these experiments varied somewhat with the techniques employed and the size of the birds.

Cobalt 60 emits two gamma rays with energies of 1.17 and 1.33 Mev. respectively, and a beta particle with a maximum energy of 0.31 Mev. It has a half life of 5.3 years. The maximum gamma radiation dose from the one or two micro-curies injected into each bird is at a rate of approximately thirteen or twenty-seven milliroentgens per hour at the site of injection assuming one centimeter distance from a point source in the cecum. As the geometry changes with time, the dose rate declines rapidly. Radiation effects are certainly minimal and probably negligible.

Preparation of Birds and Collection of Samples

Technique I:

A dozen healthy adult chickens were utilized in this experiment. The bird was secured properly on the operation table by means of a thread, tied around its legs and wings and anaesthetised.

An incision one-half to one inch long was made between the last two ribs on the left thoracic wall. A cutaneous vein runs diagonally from the wing towards the hip at the site of operation and this was carefully avoided. The intercostal muscles were incised; the peritoneum was perforated with the fingers to clearly expose the proximal ends of the cecae. The cecal tonsils were convenient landmarks for recognizing the cecae in situ. In cases of difficulty to find the cecae, a metal sound was passed through the rectum. This was a helpful guide to locate them.

The cecum was brought out by means of forceps without damaging the mesentery and a silk ligature applied on the proximal end above the cecal tonsil (ligature 1 shown in Fig. 1).

A small incision was then made on the ligated cecum below the tonsil; a suitable size and length plastic tube was introduced and secured in position by means of another silk ligature (ligature 2 shown in Fig. 1).

The little extravasated blood was mopped with sterile cotton and the ligated cecum was replaced within the abdominal cavity; the peritoneum and muscles were sewed up with interrupted sutures of chromic cat gut and the skin with continuous silk sutures. Twenty to thirty minutes was required to perform this operation and the chickens

usually recovered consciousness by this time. While the chicken was yet in the secured position, the dose of Cobalt 60 varying between two milliliters and four milliliters was injected through the plastic tube into the cecum and clamped shut. The time of injection was noted. The chicken was then released and transferred back to the cage. At periodical intervals of ten minutes, one hour, three hours, six hours, eight hours, nineteen hours, twenty-four hours, four days and one week, the operated chicken was secured on the operating table again, to collect the cecal samples. A syringe attached to the plastic tube was used for this procedure. Due, perhaps, to negative pressure in the cecum, it was difficult to obtain samples, hence it was decided to do a cecal fistula operation to open the cecal tip. This idea was developed in Technique II, well described below in Technique II.

The cecal contents were collected in preweighed, clean porcelain crucible covers for analysis. At the time of withdrawal of cecal samples, one milliliter of blood from the heart was also obtained at similar intervals of time and collected in separate porcelain crucible covers for analysis. In this technique three of the birds (birds 7, 8 and 9) were left alive for a week to repeat the same experiment to see if there was any build up of Cobalt 60

in the blood. At the end of the experiment the chickens were sacrificed by strangulation and visceral organs such as entire liver, entire spleen, entire cecum, entire cecal contents and bile from the gall bladder were collected. The cecum was cleansed in running water. All the tissue samples were placed separately in preweighed clean porcelain crucible covers for subsequent analysis.

Technique II: Three adult healthy chickens were utilized in this technique. The laparotomy was done on the left side to expose the ceca ; the cecum was ligatured at its proximal end and a suitable size and caliber plastic tube was introduced into the cecum and kept in position by another ligature. This portion of the procedure was the same as that used in technique I. The birds were usually recovering consciousness at this stage, hence a little more pentobarbitol was usually injected. The maximum dose given to a bird in this technique was 1.3 ml. of the barbiturate solution.

Following these procedures, another operation was performed for the purpose of creating a cecal fistula. A para median incision one inch long was made near the vent. The cecal tip was located by manipulation of the plastic tube already in the cecum and the cecum secured. Its tip was snipped; a glass tube of suitable size was inserted into the cecum and retained in position by means of purse

string sutures of silk (ligature 3 shown in Fig. 1). The wound was then closed aseptically; the peritoneum and muscles being sewed up with interrupted sutures of cat gut and the skin with clip sutures. The operation required thirty minutes to forty-five minutes.

Since the cecum was open at two places, it was possible to flush out the cecal contents completely. The important part of the experiment was to preserve the mesentery intact with absolutely no damage to its blood vessels or nerves. When the cecum was clean and empty, two milliliters of Cobalt 60 solution was injected into the cecum through the plastic tube on the abdominal side while the cecal tube was clamped outside. The time of injection was noted and after one-half hour, the contents were withdrawn from the cecum through the cecal tube and collected in clean preweighed porcelain crucible covers. At the time of withdrawal of cecal samples, one milliliter of blood was also obtained from the heart and collected similarly in a porcelain crucible for analysis. Only one cecal sample was obtained from chicken 1.

In chicken 2, after performing the operation described for chicken 1, the first cecal sample was collected. The cecum was flushed clean with water; injected again with two milliliter of CO 60 solution for withdrawal after

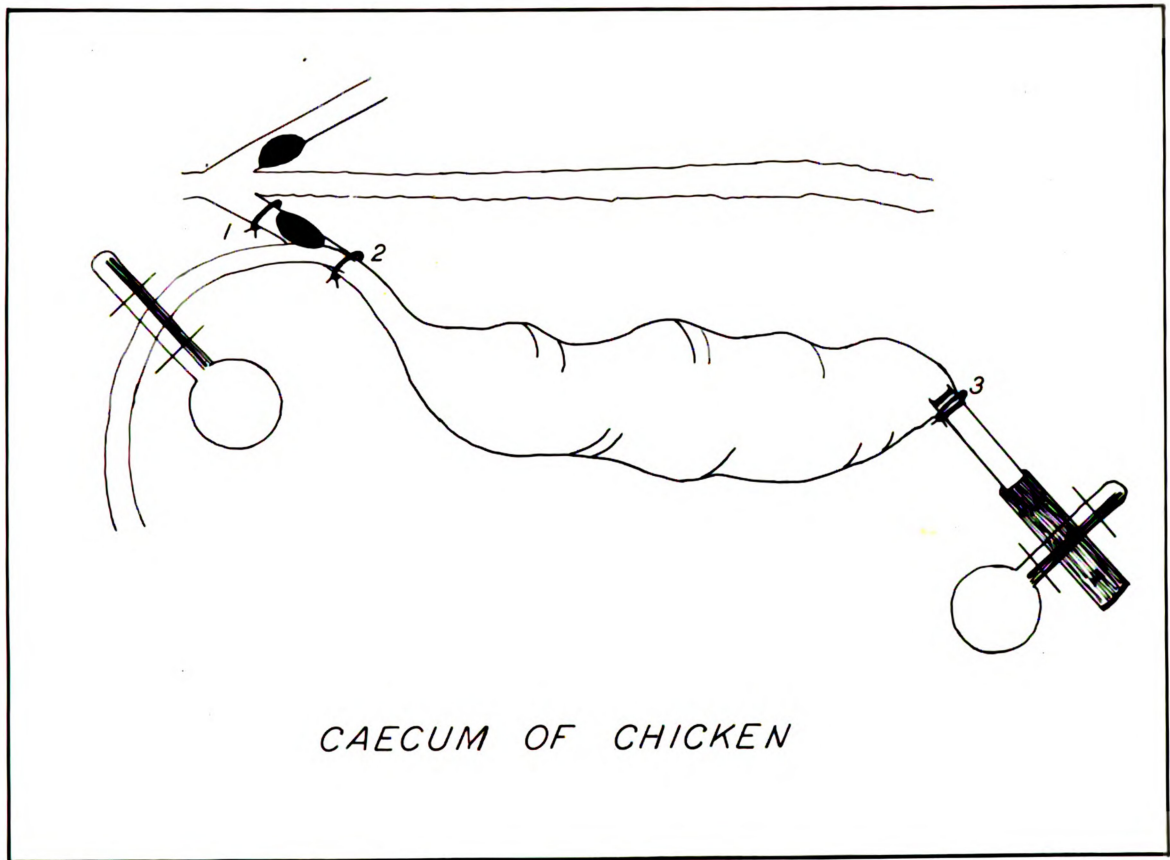


Figure 1

The cecum showing the ligatures and connections

one-half hour. The same experiment was repeated several times on the same chicken over a period of a week and twelve blood and twelve cecal samples were collected.

In chicken 3, the same operation was performed and five blood and five cecal samples were obtained. The post operative condition of all the chickens was good. The first chicken was sacrificed by strangulation after obtaining one cecal sample. The bile, liver, spleen and cecum were collected for analysis. The other chickens lived long after the strenuous operation. Besides the economy of chickens, this technique was invaluable to make a direct study of the cecal function. A total of eighteen trials were made in this technique. Eighteen blood and eighteen cecal samples were obtained for analysis.

Technique III: Twenty-nine chickens were utilized in this technique. Laparotomy was performed as described on each chicken and the cecum exposed. The proximal end was ligatured (ligature 1 in Fig. 1). No canulation or intubation of the cecum was done. One milliliter of CO 60 was injected into the cecum through the cecal tonsil. This site of injection was preferred because there was no leakage subsequent to injection. The abdominal wound was closed aseptically as was done in the previous techniques. The operation took fifteen minutes. The time of injection of

CO 60 was always noted and the chickens were sacrificed at different intervals of time of no hours, one-half hour, one hour, two hours, four hours, six hours, eight hours, sixteen hours and twenty-four hours. One milliliter of blood was obtained from the heart of each chicken at the specified intervals of time before sacrificing them. The visceral organs such as liver, bile and spleen were collected separately in preweighed, clean porcelain crucible covers. The special feature in this technique was collecting the entire cecum with cecal contents from each chicken separately into a preweighed porcelain crucible cover for analysis.

In the three techniques care was taken to preserve the mesentary intact with blood vessels and nerves. In technique I the cecum contained cecal contents; in technique II the cecum did not contain cecal contents except the injected CO 60 solution and in technique III the cecum contained cecal contents and both the cecum and cecal contents were collected for analysis.

Treatment of Samples and Determination of Radioactivity

The undried samples obtained in the three techniques were immediately weighed and recorded as the wet weight. The weight of the entire liver was taken but only a slice of tissue was retained for measuring the radioactivity, discarding the rest of the liver. The entire spleen was weighed. After obtaining the wet weights of the samples, they were transferred to an oven where they were dried at 100°C over night. Dry weights were then obtained. Dry samples were then transferred into a muffle furnace whose temperature was gradually increased to 600°C and kept at that temperature for 12 hours in order to completely ash the samples. The ashed samples were then placed in desiccators for cooling and after all of them had reached equilibrium, the ash weights were taken. The details of the weights of all the samples are given in the Appendix.

The ashed samples were then counted in a standard G-M tube-scaler unit. A single tube (Tracer Lab Model T.G. C-2, Tube Number 2AH91, a mica end window GM tube (with a window surface density of 1.9 milligrams per square centimeter) was used to count all the samples. The geometry of the counting chamber is shown in Figure 1 of the Appendix. The measured activities were corrected for background, self absorption and shelf position after which they are called "At" values,

following the terminology of George K. Schweitzer and Bernard R. Stein (1950). Detailed data upon which the self absorption and shelf corrections were based are given in the Appendix, as are the complete corrected activities (A_t) for each sample. Counting was continued in all cases until the counting error was reduced to less than $\pm$ two per cent or less.

The corrected or "true" activities (A_t , in counts per second) represent the counting rate each sample would have if corrected on shelf four and if the sample itself were weightless. These values are a summation of the counts due to the primary betas and gammas emitted, plus the counts due to secondary radiations which result from the interaction of the primary radiations with the walls of the chamber, the tube (and its housing) and the sample holder (crucible cover). Reflected radiation and "back-scatter" are counted after attenuation to varying degrees. No correction was applied for window thickness.

Since no exact study of the above-mentioned factors was made, no estimate of the proportion of the total activity due to each is possible, nor is such necessary. In general, a large fraction of the observed activity is due to beta particles. However, the "self-absorption coefficient" determined under the present conditions is only half that found by Gleason et al (1951) for pure beta counting of

Cobalt 60 under their more rigorously standardized geometry. This implies that scattered and secondary radiation of relatively high energy as well as primary gamma radiation is a much higher component of the total counting rate in the present case.

Sample activity was compared with working standards prepared from one-tenth milliliter of the injection solution, in turn standardized against a Tracer lab Cobalt 60 standard. Blood samples were counted on the fourth shelf. The cecal samples contained much more activity; hence they were counted on the eighth or ninth shelf. Surface densities (x , in milligrams per square centimeter) were measured after counting. Decay corrections were unnecessary due to the long half-life of radiocobalt (5.3 years).

Computation of the Data

The significance of the corrected or "true" activity (At) found in each sample varies with different samples. Thus At values from one milliliter of blood are concentrations, which tell us nothing of the total activity in the entire blood volume of the chicken. At values from whole spleens, however, are total quantities and not concentrations. In general, At values were first calculated as concentrations by dividing by the wet weight of the sample in grams (or by the volume in milliliters in the case of blood, bile and the injection solution). Since in most cases, wet, dry and ash weights were obtained for each sample, computations of At values per unit of water, dry solids or ash may be made from the data in the Appendix if desired.

In the case of the standard injection solution, At per milliliter of solution equals At per milliliter of water to less than one per cent error. (One milliliter of injection solution actually contained about nine milligrams of NaCl (since it was made up in physiological saline) and much less than 5 micrograms of CoSO_4 (non-active carrier). Since it contained about one half microcurie of Cobalt 60, only about 0.00045 micrograms of radioactive cobalt was present. The exact amount of inactive carrier cobalt present is not known,

but the shipment was a special lot with much higher specific activity (presumably by 10-100 fold) than earlier shipments which contained the amount of carrier cited above.)

Since injections were always into the cecum, the ratios of the true activity per gram wet cecal contents to the activity of the injected solution gives valuable information. In Technique I (cecal contents present in different amounts in different birds), after injection and mixing but before absorption, the ratio

$$\frac{\text{At}}{\text{g wet cecal contents}} \bigg/ \frac{\text{At}}{\text{ml HOH injected}} \quad \text{Ratio (1)}$$

should be less than 1.0 by the amount which the cecal contents dilute the total volume injected. For example, if two milliliters are injected and there are two grams of cecal contents, the ratio should be 0.5 (assuming a specific gravity of 1.0). If now the two milliliters of injected water are absorbed but no Cobalt 60 is absorbed, the ratio should again be 1.0. However, if no water is absorbed but cobalt is removed, the ratio should fall below 0.5. Thus Ratio (1) is an index of the relative rates of water and cobalt absorption.

If water is secreted into the cecum, the effect on Ratio (1) will be the same as that given by cobalt absorption, namely a reduction in its value. If both water and cobalt

are absorbed, the ratio might go up or down from its initial value of 0.5 (depending on relative rates of absorption) but within limits fixed by the total quantity of the cecal contents. For the example cited, the ratio could exceed 1.0 only if (a) part of the water initially present in the contents were absorbed in addition to that introduced, or (b) part of the cecal solids were additionally absorbed, or (c) some combination of (a) and (b) reduced the final volume in the cecum to less than that introduced. In each case, negligible absorption of radiocobalt is assumed. If cobalt is absorbed, there must be a corresponding decrease in total final cecal volume to raise the ratio above 1.0.

The volume of contents in the cecae examined was far from constant. Since it plays such an important part in evaluating the ratio, it is clear that only the extreme values of Ratio (1) are easily interpreted in Technique I.

In Technique II (cecal fistula open at both ends, contents flushed out and replaced with the injection solution), Ratio (1) would be expected to be slightly below 1.0 before absorption, due to the impossibility of completely draining the cecum. If the absorption rates for water and cobalt are equal, the ratio should not change. However, if water absorption is more rapid than that of cobalt under these conditions, the ratio should then increase, as with Technique I.

The technical advantage in Technique II consists in the possibility of controlling the composition of the cecal contents more adequately. Since the normal cecal contents are largely bacteria which are known to bind cobalt, Technique II and Ratio (1) provide a method by means of which the competition for cobalt between the parasites and their host may be evaluated. Although antibiotics and bacteriostatic agents were not tried in these experiments, this extension of the method is obvious.

In Technique III, the cecal contents and the cecal wall were not separately analyzed. Consequently Ratio (1) is less readily interpreted and will be ignored in the sense used above.

Cobalt Absorption Rate

It would appear probable, on the basis of the foregoing, that the average rates of water and cobalt absorption might be computed using Ratio (1) under appropriate conditions. The following considerations lead to the desired procedure (employing the simpler method of Technique 2 as a basis).

Let the total activity introduced into the cecum be designated At_i , for "true activity introduced".

$$\text{Then } At_i = \frac{At}{ml \text{ in}} \times ml \text{ in} \quad (2)$$

Similarly, let A_{to} represent the total true activity remaining in the cecum at the time of sampling.

$$\text{Then } A_{to} = \frac{A_t}{\text{ml out}} \times \text{ml out} \quad \dots \dots \dots (3) \\ \text{in Technique II}$$

$$\text{Or } A_{to} = \frac{A_t}{\text{g wet contents}} \times \text{g wet contents.} \quad (4) \\ \text{in Technique I}$$

Then the activity absorbed in the known time interval is the difference between that introduced and that remaining.

$$A_{ti} - A_{to} = \frac{A_t}{\text{ml in}} \times \text{ml in} - \frac{A_t}{\text{ml out}} \times \text{ml out} = A_{\text{tabs.}} \quad (5)$$

The percentage of the activity introduced which was absorbed is given by

$$\frac{A_{ti} - A_{to}}{A_{ti}} 100 = \% \text{ Co* Absorbed} \quad \dots \dots \dots (6)$$

Substituting appropriate values for A_{ti} and A_{to} gives

$$\left[\frac{\frac{A_t}{\text{ml in}} \times \text{ml in}}{\frac{A_t}{\text{ml in}} \times \text{ml in}} - \frac{\frac{A_t}{\text{ml out}} \times \text{ml out}}{\frac{A_t}{\text{ml in}} \times \text{ml in}} \right] 100 = \% \text{ Co* Absorbed} \quad (7)$$

Whence

$$\left[1 - \text{Ratio (1)} \times \frac{\text{ml out}}{\text{ml in}} \right] 100 = \% \text{ Co* Absorbed} \quad \dots \quad (8)$$

Whenever the quantity introduced into the cecum was two milliliters (as in Technique II) Equation (8) reduces to:

$$\% \text{ Co* absorbed} = 100 - \text{Ratio (1)} \times \text{ml out} \times 50 \quad \dots \quad (9)$$

The average absorption rate is then given by:

$$\text{Average Co* Absorption Rate} = \frac{100 - \text{Ratio (1)} \times \text{ml out} \times 50}{t} \\ \% \text{ min}^{-1} \quad \dots \dots \dots (10)$$

Where t is the time elapsed between injection and sampling. In Technique II, t was thirty minutes. Hence Equation (10) became:

$$\text{Average Co* Absorption Rate} = \frac{5}{3} \left[2 - \text{Ratio (1) x ml out} \right] \dots (11)$$

In this working equation for Technique II, Ratio (1) is easily measured with a high degree of accuracy, but the number of milliliters remaining in the cecum at thirty minutes can be determined only to a poor approximation. In fact, the exact quantitative determination of absorption rates in these experiments breaks down on this very point since an error of 0.1 ml in measuring the final volume in the cecum alters the computed absorption rate for Cobalt 60 in the present experiments by $0.123 \% \text{ min}^{-1}$, an error ranging from about six per cent with high water absorption (low recovery from the cecum) to about ten per cent with low water absorption. Since the volume measured was always lower than the true final volume, the effect is to over-estimate both the Cobalt 60 absorption rate and the rate of water absorption by as much as fifty per cent of the true value, according to the degree of success in emptying the cecum and the dead space in the cannulation system.

If Ratio (1) is measured at a time when half of the water introduced has been absorbed, Equation (11) for Technique II reduces to: $\text{Average Co* Absorption Rate} = \frac{5}{3} \left[2 - \text{Ratio (1)} \right] \dots (12)$

since the volume remaining is one milliliter. If there is reason to believe that the final volume is about a milliliter and if the actual measurement is known to be inaccurate, Equation (12) may be used as a first approximation, with a full realization of the errors involved. Calculations using Equation (12) were made. The results, however, merely emphasized the errors inherent in approximate methods.

It is important, then to find a procedure which will exactly measure the final volume remaining in the cecum. This measurement might be obtained by isotope dilution using a second tracer demonstrated to be completely non-absorbable and uniformly distributed in the cecal contents. Chromium 51 was obtained for this purpose in view of the work with chromic oxide for determining the non-digestible residue in forages. Since it proved to be absorbable from the cecum, its use had to be abandoned, however.

Considerations similar to these outlined earlier lead to another exact expression for describing the rate of cobalt absorption in Technique I where cecal contents were present when the cobalt solution was introduced. An equation similar to Equation (7) may be set up employing Equation (4) instead of Equation (3). Thus:

$$\% \text{ Co* Absorbed} = \left[\frac{\frac{At}{ml \text{ in}} \times ml \text{ in} - \frac{At}{g \text{ wet contents out}} \times g \text{ wet contents out}}{\frac{At}{ml \text{ in}} \times ml \text{ in} - \frac{At}{ml \text{ in}} \times ml \text{ in}} \right] 100 \quad (13)$$

Which reduces to:

$$\% \text{ Co* Absorbed} = 100 - \text{Ratio (1) x g wet contents out x 50} \quad \dots \dots (14)$$

if the volume injected was two milliliters, or to

$$\% \text{ Co* Absorbed} = 100 - \text{Ratio (1) x g wet contents out x 25} \quad \dots \dots (15)$$

if the volume injected was four milliliters.

Equations (13), (14) and (15) are the counterparts of Equations (7) and (9). Exact equations which are the counterparts of Equation (11) may then be set up for the various sampling times and quantities injected in Technique I.

Again, the technical difficulties of determining the total quantity of cecal contents present at the end were not surmounted but recourse cannot be made to a uniform value in setting up approximate equations. Unlike the impression gained in Technique II that the final volume was reasonably fixed to a first approximation, in Technique I, it was apparent that it was highly unreasonable to expect all chickens to have cecae of the same relative size, each filled at all times with the same quantity of contents.

Accordingly, two approaches were followed. First, the exact formulae were employed as though the weights of the cecal contents recovered were valid, and the data analyzed. Second, Technique 3 was instituted as an

alternative method. In Technique I, if the cecal contents were firm, it was virtually impossible to secure all but a small sample by aspiration. In some cases, none could be obtained. In all cases, after various attempts to remove the entire contents, the bird was finally killed and the contents scraped from the cecal wall. Although this last procedure was the best, it still left a large portion of the activity on the mucosa or in its crypts. Washing the cecal wall was attempted, but this obviously eliminated the possibility of obtaining the weight of the adherent contents. In Technique 3, these attempts to secure entire contents alone were abandoned and a frank estimate of the activity of "contents plus cecal wall" per weight of "contents plus cecal wall" was substituted.

The rate computations in the last case, although possible, are not informative and are subject to misinterpretation. Where the quantity of cecal contents at any time is important to the rate calculation but the quantity has not been satisfactorily estimated, the arithmetic is best abandoned. Accordingly, recourse is made primarily to semi-quantitative comparisons between the amount of cobalt taken up by the tissues or by the blood as an inversefunction of the activity per unit weight of cecal contents. It is reasoned that if

a gram of cecal contents contains a high amount of activity, the amount of activity in the tissues of the host should probably be reduced.

Tissue levels are expressed both as concentrations, At per gram wet tissue, (1., as a percentage of the concentration in the injected dose and 2., as a percentage of the blood concentration) and as the total amount present in entire organs, At per organ, (as a percentage of the injected dose).

In the light of the above mentioned efforts to evaluate the quantitative aspects of cecal function, it is obvious that the obstacles were not computational but technical. Although Techniques I and III gave valuable qualitative data, Technique II is essential for quantitative work. With this technique, the further difficulties should not be unsurmountable.

Water Absorption Rate

The rate of water absorption is given for Technique II by:

$$\text{Average HCH Absorption Rate} = \frac{\text{ml in} - \text{ml out}}{\text{ml in}} \times \frac{100}{30} \dots (16)$$

Which reduces to:

$$\text{Average HCH Absorption Rate} = \frac{5}{3} (2 - \text{ml out}) \% \text{ min} - 1 \dots (17)$$

The comparable equation for cobalt absorption has been shown to be:

$$\text{Average Co* Absorption Rate} = \frac{5}{3} (2 - \text{Ratio (1) ml out}) \dots (11)$$

from which it may be shown that the relative rate of cobalt to water absorption in Technique II is given by the ratio:

$$\frac{\text{Average Co* Rate}}{\text{Average HOH Rate}} = \frac{1 - \frac{\text{ml out}}{2} \text{ Ratio (1)}}{1 - \frac{\text{ml out}}{2}} \quad \text{Ratio (2)}$$

From Ratio (2), it is evident that if Ratio (1) is 1.0, Ratio (2) will also be 1.0 indicating that water and cobalt are absorbed at the same rates (in % min⁻¹). It is also evident that if Ratio (1) is less than 1.0, the cobalt absorption rate will exceed the water absorption rate, as indicated earlier.

It is also apparent that if one can determine only two variables, namely the volume of fluid remaining in the cecum (i.e. "ml out") and the value of Ratio (1), it is possible to compute the absorption rates for both the solvent and the solute used.

Similar considerations lead to the following equation for the rate of water absorption from the cecum in Technique I (if two milliliters were injected and t is thirty minutes):

$$\text{Average HOH Absorption Rate} = \frac{5}{3} (2 - \text{ml present} - \text{ml out}) \% \text{ min}^{-1} \dots (18)$$

where ml present = total water in cecal contents before injection
ml out = total water in cecal contents at the end.

Equation (18) obviously cannot be solved from data obtained in the present experiments. In this case, other useful approximations are possible. If it is assumed that no water was initially present, the results computed from the data will be distorted, of course, but the values obtained will be close to the minimal limits for the rate of water absorption.

A "maximal" rate of water absorption may be computed on the assumption that no cobalt is absorbed. In that case, Ratio (1) would reflect concentration of the injected solution by the withdrawal of water alone. The appropriate equation is (for two milliliters injected and a time of thirty minutes):

$$\text{Average HOH Absorption Rate} = \frac{2 - \frac{1}{\text{Ratio (1)}}}{2} \frac{100}{30} \dots (19)$$

which reduces to

$$\text{Average HOH Absorption Rate} = \frac{5}{3} \left[2 - \frac{1}{\text{Ratio (1)}} \right] \% \text{ min}^{-1} \dots (20)$$

In Equation 20, the reciprocal of Ratio (1) is the quantity of water necessarily remaining at the end of the time period if the injected cobalt is present and concentrated to the extent found. Equation (20) does not give a true maximum, since (a) if cobalt is absorbed at all, Ratio (1), as measured, will be lower than otherwise and (b) Ratio (1) is computed from At per gram of cecal contents, not from At per ml finel

water found. Both of these considerations reduce the value computed for the approximate absorption rate.

Equations (18) and (20) then should give two estimates for the rate of water absorption in Technique I. Equation (18) gives values that should be too low, Equation (20) gives values that should probably be too high. The true rate might then fall at someplace between.

This rather extended discussion is significant insofar as it outlines the conditions under which further attempts to measure cecal absorptive function (or for that matter, uptake from any external solution) are likely to lead to quantitative results. Qualitative conclusions may be obtained much more simply but only if certain types of experimental findings appear.

DATA AND RESULTS

The actual results obtained in Technique I are given in Table 1. It will be noted first, that Ratio (1) is generally much greater than 1.0, indicating that water was absorbed more rapidly than cobalt. Seventeen out of the twenty-one trials gave this result. However when the cobalt and water absorption rates are actually computed from the data, no marked differences appear. This is entirely due to the inadequacy of the values in Column 3 (g. wet contents out) which represent only a small and variable fraction of the total cecal contents present at the stated times after injection of cobalt solution.

Table 1

Apparent Cobalt and Water Absorption Rates in Technique I

(rates in apparent % min⁻¹.)

Time Interval	ml in	g Wet Contents Out	Ratio (1)	Co* Abs Rate	H ₂ O min.	Abs Rate "max."
10 m	4	.3068	1.74	8.665	9.253	8.563
60 m	4	.5153	1.17	1.415	1.477	1.311
60 m	4	.4748	1.15	1.439	1.482	1.304
60 m	4	.3404	2.66	1.289	1.538	1.510
60 m	4	.5505	1.25	1.380	1.447	1.333
60 m	4	.2734	3.32	1.288	1.561	1.541
60 m	4	1.0018	1.50	1.041	1.274	1.389
Average 60 m		0.5260	1.84	1.309	1.463	1.398
180 m	4	.4093	1.02	0.498	0.502	.419
180 m	4	.3397	1.23	0.498	0.513	.443
Average 180 m		0.3745	1.12	0.498	0.508	0.431
360 m	2	.6161	1.92	.113	0.214	.205
480 m	2	1.799	0.59	.098	0.084	.032
1140 m	4	.2754	1.35	.080	0.082	.080
1440 m	2	1.9121	0.68	.024	0.021	.019
1440 m	2	3.1577	0.51	.017	-0.014	.001
1440 m	4	.3349	2.40	.055	0.065	.062
1440 m	4	.4626	2.12	.052	0.063	.061
1440 m	4	.8635	2.05	.039	0.059	.061
Average 1440 m		1.3461	1.55	.037	0.044	.041
5760 m	4	1.0737	1.24	.012	0.014	.014
5760 m	4	.5143	0.03	.017	0.015	-.012
Average 5760 m		0.7940	0.63	.015	0.015	0.001
10080 m	4	1.131	1.35	.006	.008	.008
10080 m	4	.573	1.46	.008	.009	.008
Average 10080 m		.852	1.41	.007	.009	.008

Table 2, lists cobalt absorption rates computed from the data of Technique III. These values are considerably lower. It appears as though with Technique I, cobalt absorption rates, calculated from the available data, are at least fifty per cent too high, due to the incomplete recovery of cecal contents. There can be no doubt however, that shortly after its injection, cobalt is rapidly absorbed but after a few hours, very little additional is removed, despite the fact that large amounts still remain in the cecum.

Table 2
Cobalt Absorption from the Cecum
at the Different Time Intervals in Technique III

	$\frac{1}{2}$ hr	1 hr	2 hrs	4 hrs	6 hrs	8 hrs	16 hrs	24 hrs.
% Co 60 Present	81%	75%	71%	76%	65%	79%	66%	70%
% Co 60 Absorbed / min.	0.633	.417	.242	.100	.097	.044	.035	.021

In Table 3, are recorded the rates of cobalt and water absorption computed from the data of Technique II. It will be seen that the Ratio (1) is generally less than 1.0, indicating that cobalt is absorbed faster than water. Seventeen out of eighteen ratios (Ratio 1) gave this result.

Table 3

Cobalt and Water Absorption Rates in Technique II

t = 30 min ml in = 2 rates in % min⁻¹

Data	ml out	Ratio (1)	Co* Abs Rate	HON Abs Rate
Bird 1	1 ml	0.32	2.80	1.7
Bird 2	1 ml	0.67	2.22	1.7
	1.7	0.74	1.24	0.5
	1.5	0.82	1.28	0.8
	1.6	0.57	1.81	0.70
	1.6	0.68	1.52	0.7
	1.7	0.86	0.90	0.5
	1.8	0.59	1.56	0.3
	1.3	0.93	1.32	1.2
	1.3	0.69	1.84	1.2
	1.2	0.74	1.85	1.3
	1.5	0.79	1.36	0.8
	1.3	<u>0.68</u>	<u>1.86</u>	<u>1.2</u>
Average Bird 2		0.73	1.56	0.66
Bird 3	1.3	0.86	1.37	1.17
	1.0	1.10	1.5	1.7
	1.8	0.86	0.75	0.33
	1.0	0.68	2.2	1.17
	1.0	<u>0.75</u>	<u>2.08</u>	<u>1.17</u>
Average Bird 3		0.85	1.58	1.11

Attention should be directed to the relative rates of cobalt absorption observed in Technique III (cecal contents present, Table 2) and in Technique II (no cecal contents, Table 3). In the presence of material in the cecum, at thirty minutes, the average cobalt absorption rate was $0.633\% \text{ min}^{-1}$. With no cecal contents, the thirty minutes absorption rate for cobalt was found to vary between 0.75 and $2.8\% \text{ min}^{-1}$. This is direct evidence indicating that the presence of normal cecal contents reduces the rate of cobalt uptake from the cecum to about half of the value found in the absence of such materials. Furthermore, after as long a period as twenty-four hours, in the presence of cecal contents, seventy per cent of the injected cobalt remained unabsorbed. Thus it is not a case in which there is only a slowed absorption rate but with ultimately, all of the cobalt being removed. The cobalt is also bound in some way so that a large fraction of it (up to seventy per cent) can be absorbed only with extreme slowness. It is logical to assume that the cobalt is bound in bacterial cells.

The absorption rates for cobalt and water are graphically shown in the figures. Figure 2, drawn from the data of Technique I, indicates the computed rates of absorption for the cobalt (a maximal value due to the fact that not all the cecal contents were removed) and for water (a minimal value

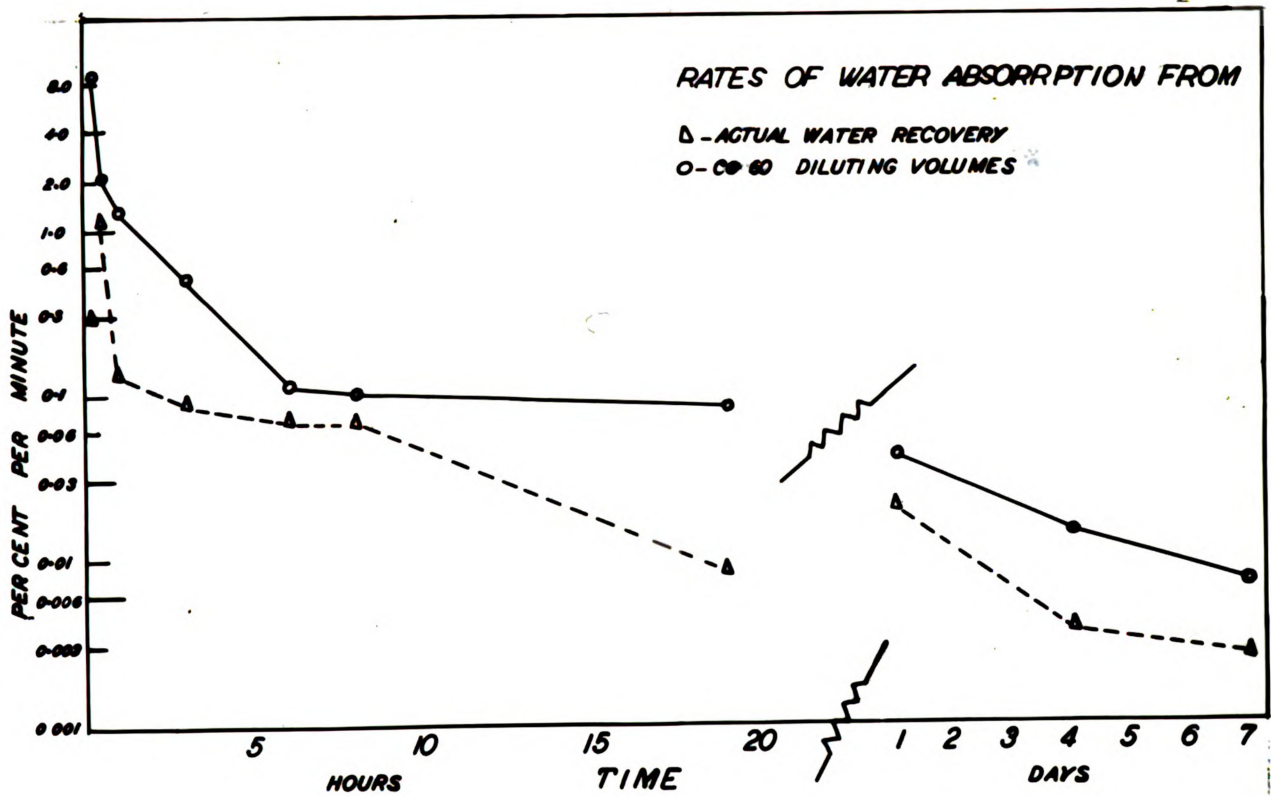


Figure 2

The Percentage Rate of Cobalt and Water Absorption

* Cobalt 60

due to the fact that water already present in the cecum before injection, was not taken into account and that complete removal of the cecal contents was technically difficult).

The approximate percentage of cobalt absorbed at a half hour by chickens (Technique II) in eighteen trials are recorded in Table 4.

Table 4

Percent Cobalt Absorbed at $\frac{1}{2}$ Hour from the Cecum

Birds	Trials												Ave. %
	1	2	3	4	5	6	7	8	9	10	11	12	
1	85%	-	-	-	-	-	-	-	-	-	-	-	
2	67%	63%	59%	72%	66%	57%	71%	54%	66%	63%	60%	66%	63%
3	57%	45%	57%	66%	63%	-	-	-	-	-	-	-	
Averages:	(1) 85%				(2) 64% $\pm$ 5				(3) 58% $\pm$ 3				

These data are computed using the approximate Equation (12). It will be seen that an average of 63% of cobalt was absorbed in half an hour. These results are higher than those listed in Table 3 and serve to illustrate the errors introduced by the use of an approximate method of computation. The approximate results are graphed in Figure 3.

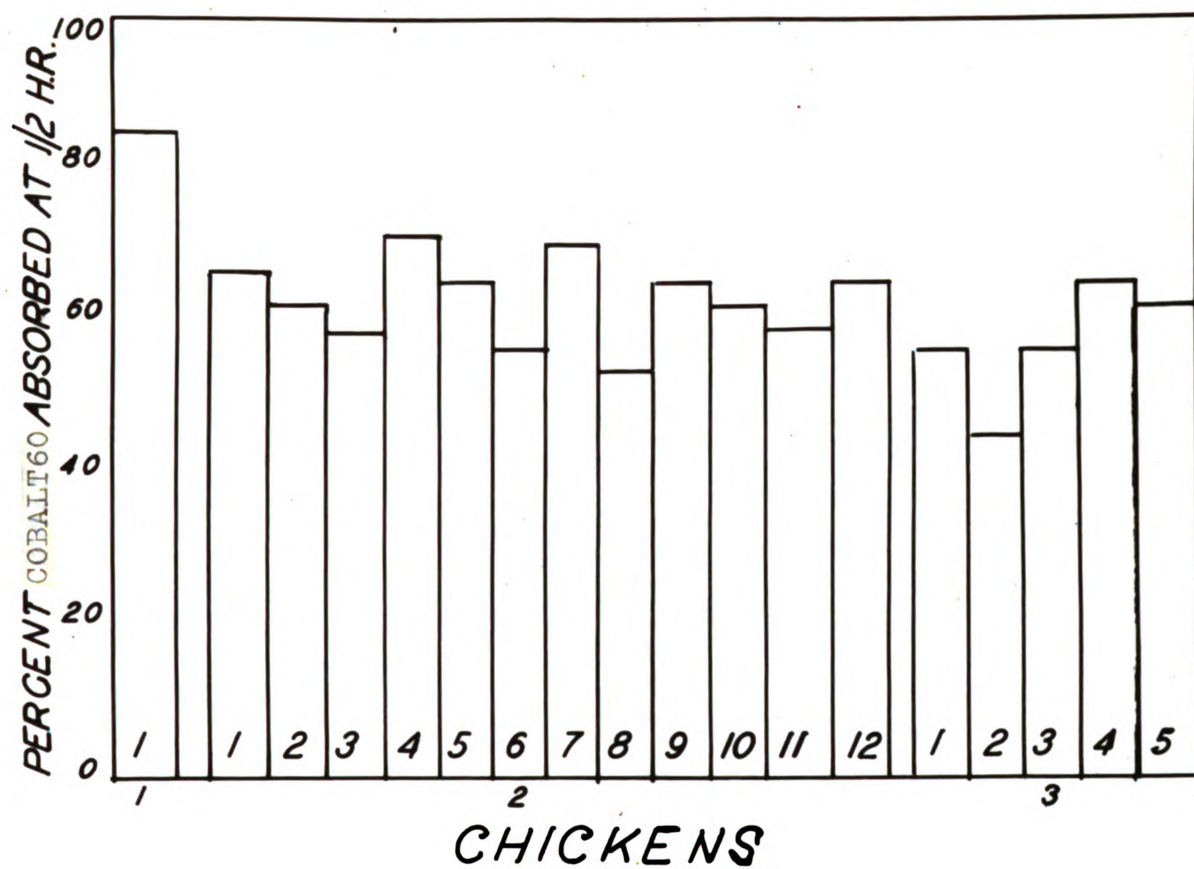


Figure 3
Percentage of Cobalt 60 Absorbed at Half Hour from the Cecum

Evidence for cobalt absorption is also given by finding cobalt in the blood and visceral organs such as the liver, spleen and cecal wall, and also in the bile. The per cent concentrations of Co 60 in one milliliter of blood at different times in chickens (Techniques I and II) are recorded in Table 5.

Table 5

Percent Concentration of Co 60 in Blood
at Different Intervals of Time in Techniques I and II

	Intervals								
	3 hrs	6 hrs	8 hrs	10 hrs	20 hrs	24 hrs	25 hrs	26 hrs	1 week
Tech I	0.233	0.180	.316	.051	.074	.072	.134	.317	.033
Tech II	18 trials $\frac{1}{2}$ hour interval -- 0.204% in ml of blood								

The fluctuating blood values observed at different times may perhaps be explained by the different quantities of cecal contents present in the different birds killed at different times. These data are graphically shown in Figure 4. The line drawn in Figure 4 is plotted from the data of Monroe et al (1952). It shows the concentration of cobalt in blood when 1.0 micro-curie of Cobalt 60 was injected intro-peritoneally into chickens. The unconnected points in the figure represent the blood concentrations found in the present experiments, where Co 60 was injected into the cecum. It is evident from

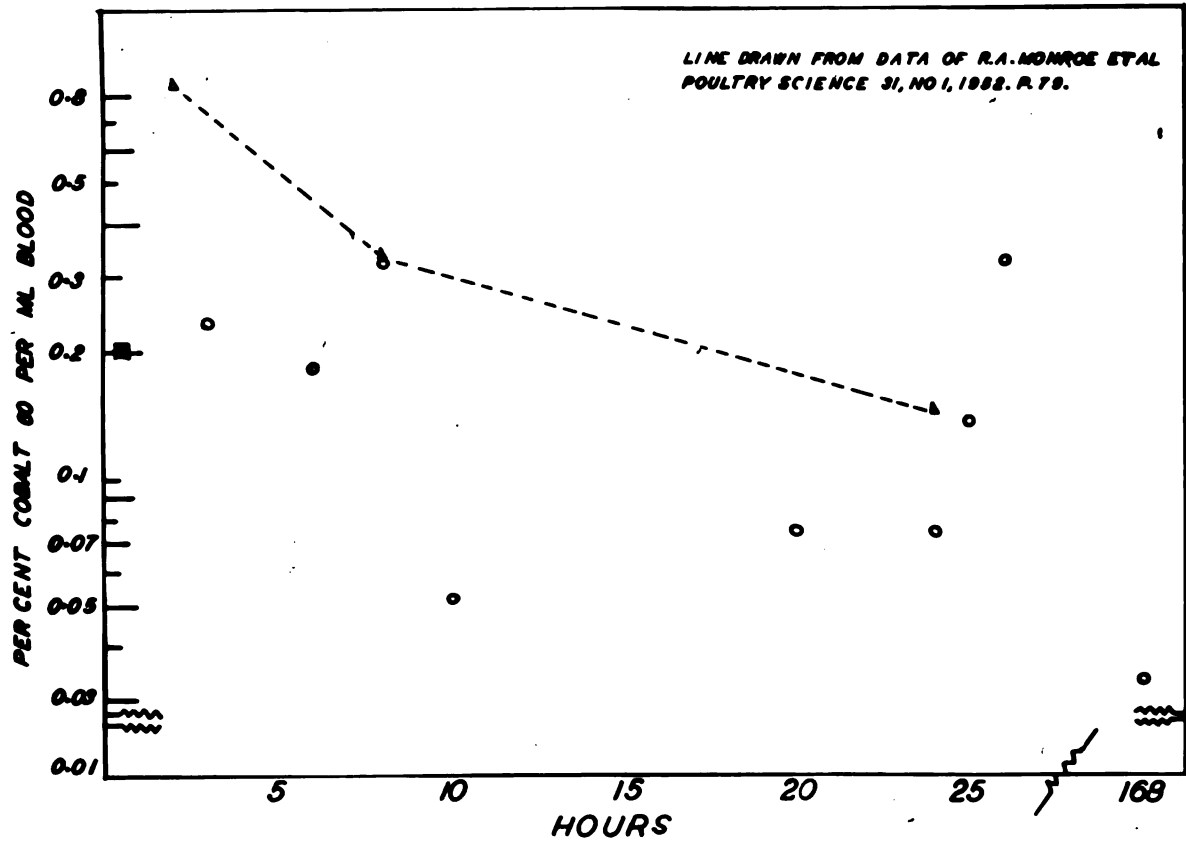


Figure 4

The pick up and turn over of Cobalt 60 in blood

the figure that the uptake of Cobalt 60 was lower following intra-cecal injections than has been reported following injection into the peritoneum. This again may be due to the binding of cobalt by the cecal contents.

The average percentage tissue concentrations of cobalt in the visceral organs are recorded in Table 6 and the total quantity of Co 60 present in the entire organs are recorded in Table 7.

Table 6

Average Percent Tissue Concentration of Co 60
at Different Times

Expressed as Ratio (1) $\frac{\text{At}}{\text{g wet wt}} / \frac{\text{At}}{\text{ml injected}} \text{ times } 10^2$

Tissues	Intervals						
	$\frac{1}{2}$ hr	6 hrs	8 hrs	16 hrs	24 hrs	4 d	1 week
Liver (1)	0.085(4)	0.087(2)	0.094(4)	0.027(7)	0.078(1)	0.055(2)	0.053
Bile (1)	0.302(4)	0.491(2)	1.16 (4)	0.322(4)	0.173(1)	0.292(1)	0.056
Spleen(1)	0.037(1)	0.937(1)	0.048	- (4)	0.045(1)	0.017(2)	0.023
Cecal Wall (1)	0.387(1)	4.980(1)	1.520	- (6)	1.298(1)	5.270(1)	1.070

Table 7

Average Total Percent of Co 60 Present in Entire Organs

Liver (1)	5.025	-	-	- (6)	3.268(1)	2.681(2)	1.842
Spleen(1)	0.104(1)	0.477(1)	0.058	- (4)	0.090(1)	0.069(2)	0.061
Cecal Wall (1)	1.861(1)	7.065(1)	3.076	- (6)	4.107(1)	2.234(1)	3.562

These results were obtained from the chickens in the Techniques I, II and III. The values obtained from the cecal wall itself, were from Techniques I and II since in Technique III, the cecal wall was not separately analyzed. The number of birds from which the samples were obtained is also recorded in the same tables.

The concentration ratios of Co 60 to blood in the visceral organs such as liver, bile, spleen and cecal wall are recorded in Table 8.

Table 8

Ratio of the Concentrations of Co 60 in the Tissues
to that in Blood (Apparent Cobalt "Spaces")

Tissues	Intervals						
	$\frac{1}{2}$ hr	6 hrs	8 hrs	16 hrs	24 hrs	4 d	1 week
Liver (1)	0.195(4)	0.697(2)	0.542(4)	0.563(7)	0.883(1)	0.172(2)	1.350
Bile (1)	0.692(4)	4.468(2)	24.760	- (4)	1.960(1)	0.924(1)	1.690
Spleen(1)	0.086(1)	2.770(1)	0.153	- (4)	0.414(1)	0.052(2)	0.600
Cecal Wall (1)	0.892(1)	14.70(1)	4.800	- (6)	13.100(1)	5.810(1)	32.200

The figures in Table 8 represent the number of milliliters of blood which contain the amount of cobalt present in one gram of wet tissue. For example, at six hours, one gram of fresh liver contained an

amount of cobalt equal to that found in about 0.7 milliliter of blood. Since there is never that amount of blood in a gram of liver, it is obvious that at least some of the cobalt must have entered the liver cells. These ratios change with time in a manner peculiar to each tissue. Although there are not enough samples to completely analyze the time-activity curves for each tissue separately, it is clear that much of the cobalt which entered these tissues in the first half hour or so when the blood level was high is lost later. Since the ratios at one week are still high, apparently cobalt is lost from the blood more rapidly than from the tissue cells.

In Table 9, are recorded the values for the percentage concentrations of Cobalt 60 in a milliliter of blood, the average concentration ratio for Co 60 in the cecal contents to blood, and the wet weights of cecal contents at the different times.

Table 9

Percent Concentration of Co 60 in Blood and Its Relation to the Quantity of Cecal Contents at Different Intervals of Time

	Time Intervals								
	0	$\frac{1}{2}$ hr	1 hr	2 hrs	4 hrs	6 hrs	8 hrs	16 hrs	24 hrs
% Co60 in ml blood	.215	.241	.059	.062	.139	.064	.053	.175	.049
Ave. conc. ratio in Cecal con.	224	209	384	406	274	458	415	378	414
Wet wts.	4.447	3.248	3.832	3.833	2.671	5.879	4.398	4.046	5.663

These values were obtained exclusively from the twenty nine chickens utilized in Technique III. The results are shown graphically in Figure 5. It is evident that the activity in blood varies inversely with the weight of the cecal contents, (that is, the activity in the blood decreased when the wet weights of cecal contents increased and vice versa). This indicates that more cobalt is bound when the total quantity of cecal contents is high than if few contents are present.

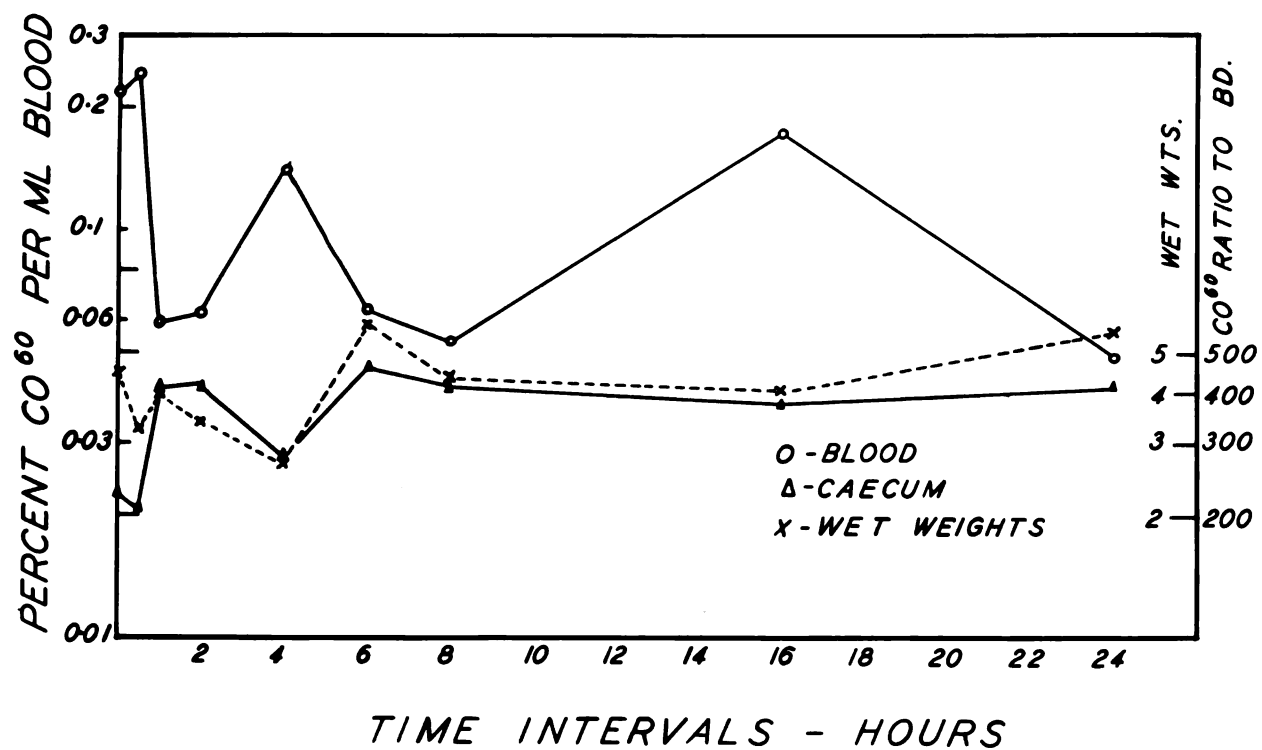


Figure 5
Radioactive Cobalt 60 in Blood and Its Relation to the
Cobalt 60 Present in Cecum of Chickens

The foregoing has been concerned primarily with the evidence for cobalt absorption and binding by the cecal contents. Water is also absorbed, as has been indicated directly in Table 3 and implied in the consideration of Ratio (1). That the rate of water absorption also declines with the passage of time after injection may be seen even for the technically unsatisfactory data from Technique I listed in Table 1. From Table 9, it may be seen that after the interval of a day, the average weight of cecal contents is no less than at the beginning. Much of the weight is water (see Appendix for numerous examples). Therefore, all of the water cannot be removed any more than can all of the cobalt. This implies that water too is bound.

Is the initial rate of water uptake modified by the presence of cecal contents? The data reported herein are not satisfactory on this point. In the absence of cecal contents, the average rate at one half hour after injection was in the neighborhood of one per cent per minute. In the presence of cecal contents, the average rate at one hour after injection, based on unsatisfactory data, was computed to be about 1.4 per cent per minute. If this last figure is fifty per cent high (a not unreasonable assumption), a more correct value might be about 1.0 per cent per minute;

in other words, indistinguishable from the value obtained in the absence of cecal contents. Thus the present data provide no basis for concluding that the bacterial flora inhibit the initial rate of water absorption from the cecum. The data, however, are far from adequate with respect to this point.

Special Observation

In chicken 10 of Technique I the cecal tube was accidentally cut asunder below the cecal tonsil while doing the laparotomy. The cecum was then canulated without the cecal tonsil being present. Interestingly enough, it was possible to withdraw a greater amount of the sample from the cecum of this bird than from another chicken with the cecal tonsil intact operated similarly on the same day.

It is doubtful if the cecal tonsil itself has any function in water absorption since it is reported to be nothing but a mass of lymphocytes held together in a framework of reticular cells (Fulton, T. F. 1949). But the tonsillar area appears to be important in the function of water absorption, in view of the findings of Calhoun, M. Lois (1933) and others who have observed a greater number of villi present in this area. Therefore it appears that in the absence of the absorptive area covering the cecal tonsil, water is not fully absorbed. Hence it was possible

to withdraw a greater amount of the injected sample from the cecum ligated so as to exclude the "tonsil." It is clear that the cecal tonsil itself probably does not serve any function in water absorption though a large portion of the total absorption may occur in the region of the cecal tonsil. This is, however an isolated and accidental observation in a single chicken.

DISCUSSION

The results of these exploratory experiments constitute the first direct evidence that water is absorbed by the cecum of birds. This finding was not unexpected, however, in view of the indirect evidence of Mangold, Radeff, Keith et al, Browne and Olson et al cited earlier. The rate at which water enters the cecum from the intestine is not known but it probably does not enter in a steady but slow stream. The data presented above indicate that if, for example, a milliliter entered, it would probably be absorbed in about one hour. This might then be repeated as often as "free" water was present in the lower small intestine. Together, then, the two cecae might absorb as much as forty or fifty milliliters of water each day, if they are constantly supplied with water. This is a significant amount in an animal weighing about two kilograms. Whether they actually absorb this amount (or even more) is not known. It is clear, however, that loss of the cecal water, due to defective absorptive function by disease or parasitism might well be noticed first by finding an increased amount of water in the litter on which the birds are kept.

However, the quantity of bacteria in the cecum (or more exactly, the weight of the cecal contents) was not shown to influence the initial rate of water absorption. If water

is absorbed primarily in that portion of the cecum nearest the intestine, this might not be unexpected. The relation of bacteria or other cecal parasites to water absorption (as well as to absorption of other materials) requires further investigation particularly in terms of the total water balance of the organism.

The results clearly indicate that cobalt can be absorbed from the cecum but that, in effect, the cecal contents (presumably the bacteria present) compete with the chicken for this element. Under the conditions studied, the contents win out. Shirley, R. L. et al (1952) have reported the excretion both of P³² and Ca 45 into ligated cecae. Lee and Wolterink have found from this laboratory (data in press) that Co 60 is also secreted into the ligated cecum after injection into the gizzard lumen. Thus cobalt can go either way. In view of the remarkable affinity of many bacteria for cobalt, demonstrated indirectly here and directly by Chow, E. F. (1951) in vitro, it is apparent that competition for trace elements may seriously affect the economy of the bird.

In the case of cobalt, the bacteria presumably use at least a part of it to manufacture vitamin B₁₂. If this vitamin is liberated by the bacteria into the lumen of the

cecae and if it can subsequently be absorbed by the bird, a degree of symbiosis may occur which is advantageous to both. If however, the bacteria constitute a sink into which is drained the bird's supply of cobalt and if they are expelled along with their vitamin B 12 when the cecum empties, the maintenance of a cecal population of bacteria might be luxury which the bird might not always be able to afford.

If vitamin B 12 containing Cobalt 60 was formed in these experiments, an appreciable amount of it might have been absorbed from the ligated cecum in twenty-four hours. In Technique III (see Table 2), the cecum plus its contents contained about 80 per cent of the injected dose at one half hour after injection. At twenty-four hours after injection it still contained about 70 per cent of the injected dose. From these figures, assuming that all of the 80 per cent was used to manufacture vitamin B 12 and that the 10 per cent which was then slowly absorbed was entirely vitamin B 12, as much as 10.0 - 100.0 micrograms of the vitamin might have been absorbed (10% of the 0.005 - 0.05 micrograms of cobalt injected = .5 - 5.0 micrograms of cobalt. Since one microgram of cobalt equals about twenty micrograms of vitamin B 12, the 10% of cobalt absorbed equals about 10.0 to 100.0 micrograms of B 12 absorbed)

This calculation merely indicates that the radiocobalt data are not inconsistent with the idea that the cecal bacteria may play a prominent role in supplying vitamin B 12 to the chicken. Again, more exact studies are indicated.

The fact that cobalt can apparently be absorbed even more rapidly than water requires some comment. This finding cannot be explained by any combination of diffusion mechanisms. It necessitates the postulation of a highly efficient and probably specific physiological mechanism for removing cobalt from solution. That such mechanisms exist is also implied by the findings of Lee and Wolterink (unpublished data, this laboratory) that the liver is able to concentrate cobalt in bile to an astonishing extent. The kidney is also able to concentrate this element in urine beyond the degree explainable on the basis of water reabsorption alone. The possibility that the cecum is able to "exclude" water for the plasma, that is, to take up less water than might be expected, must always be considered. The mechanism behind this observation is completely unknown.

SUMMARY AND CONCLUSION

Experiments have been conducted to evaluate the function of the cecum in chickens with respect to the absorption of water and Cobalt 60.

Three different Techniques were employed, utilizing forty-four healthy chickens of varying ages, sexes and weights.

Results obtained from the three Techniques demonstrate that:

1. The cecum absorbs water.
2. The cecum absorbs Cobalt.
3. Cobalt is absorbed faster than water in the absence of cecal contents but less rapidly than water where the contents of the cecum are present.
4. Cobalt is bound in large amounts by the cecal contents.
5. A certain fraction of the water also remains bound to the cecal contents.

The possible role of the cecal tonsil is discussed.

It is hoped that the procedures evolved will be helpful especially in further studies of bacterial binding with special reference to various drugs.

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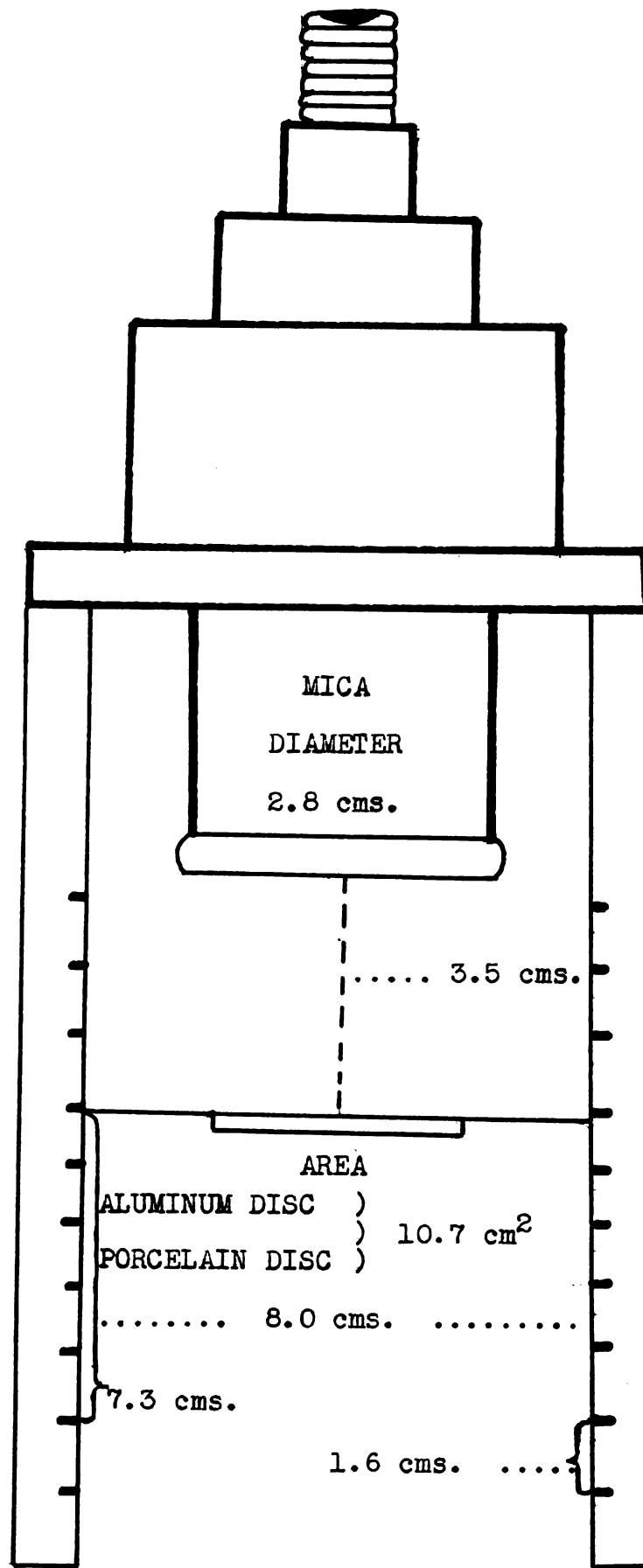
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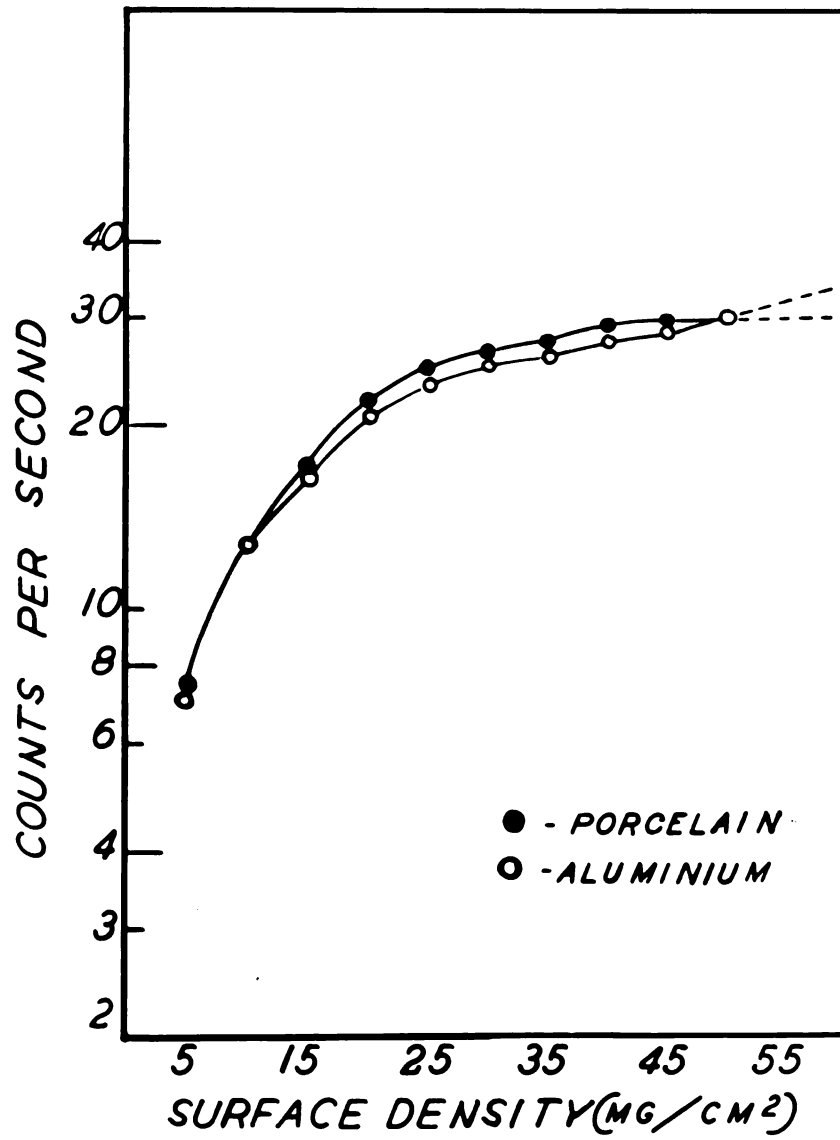
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APPENDIX

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Geiger Mueller Tube with the Counting Shelf
Showing the Geometry of the Counting Arrangement
in Measuring the Radioactivity of Samples



A Curve Showing the Self-Absorption
of Cobalt 60

SELF-ABSORPTION CORRECTION FACTORS FOR THE LOSS OF RADIOACTIVITY
DUE TO THICKNESS OF THE SAMPLES

		Surface Densities (mg/cm ²)									
		.0	.1	.2	.3	.4	.5	.6	.7	.8	.9
1	.0431	.0044	.0088	.0131	.0175	.0218	.0261	.0304	.0346	.0389	
2	.0843	.0473	.0515	.0557	.0598	.0639	.0681	.0721	.0762	.0803	
3	.1238	.0883	.0924	.0963	.1003	.1043	.1083	.1121	.1160	.1199	
4	.1615	.1276	.1315	.1353	.1391	.1429	.1467	.1504	.1541	.1580	
5	.1977	.1652	.1689	.1726	.1762	.1798	.1834	.1870	.1906	.1941	
6	.2322	.2012	.2047	.2082	.2117	.2152	.2186	.2220	.2255	.2289	
7	.2653	.2356	.2390	.2423	.2457	.2490	.2523	.2556	.2588	.2621	
8	.2970	.2686	.2718	.2750	.2782	.2813	.2845	.2876	.2908	.2939	
9	.3273	.3001	.3033	.3078	.3093	.3123	.3153	.3183	.3217	.3086	
		.3302	.3332	.3361	.3391	.3419	.3448	.3477	.3506	.3534	
10	.3562	.3840	.4106	.4360	.4603	.4835	.5058	.5271	.5475	.5670	
20	.5856	.6035	.6206	.6369	.6526	.6675	.6819	.6957	.7087	.7212	
30	.7333	.7447	.7558	.7663	.7763	.7860	.7952	.8040	.8125	.8205	
40	.8283	.8357	.8428	.8496	.8560	.8622	.8711	.8739	.8793	.8844	
50	.8895										

Self Absorption Experiment

Twenty-five chickens, all weighing less than $\frac{1}{2}$ k.g were sacrificed to obtain the entire cecae from all of them. A total quantity of 7 cc of Co 60 (activity 0.604uc/cc) was introduced, a little into every cecum, to distribute Co 60 solution uniformly in the tissue sample. The cecae were dried in the oven at 100°C over night and the dried samples were then transferred to the Muffle Furnace for ashing at 600°C being kept at that temperature for twelve hours. The ashed cecae was cooled; ground into fine powder and cecal ash was distributed evenly in porcelain crucible covers and aluminum discs. The ash bulk was piled in the receptacles in different surface densities varying from 5 mg / cm² to 50 mg / cm². The radioactivity in these samples were measured by placing all the samples in the eighth shelf of the counting stand under the thin window of the Geiger Mueller Tube. The activity was obtained in terms of counts per second for the different surface densities and the values were plotted on a semi-log paper to describe the self absorption curve shown in Appendix.

Am values were obtained for the different surface densities and according to the formula of Schweizer and Stein (1950), the absorption coefficient (b/r) and the correction factors (Am/Ai) derived from it were calculated. Am is the observed activity and Ai is the activity at infinite thickness of the cecal ash.

These factors are given in Appendix (page 3).

From the A_i values, A_t values were computed by the following formula:

$A_t = A_i 0.044 \cdot x$, where A_t is the true radioactivity without self absorption. A_i , the activity at infinite thickness, 0.044 is the self absorption coefficient obtained in the experiment and x , the surface density, (mg/cm^2) of all the radioactive samples.

The actual values obtained in the experiment and adopted in the calculations are as follows:

$$A_i = 35$$

$$b/r = 0.044$$

$$b = 4.2 \text{ if } R (\text{max range}) = 94.7 (\text{mg}/\text{cm}^2)$$

Scaler No. P.P 846
G-M Tube No. 2. A.H. 91
Operating Voltage - 1250 V.

Subject: Standard Counts
at Different Shelves.
Background: 0.813 Cts./Sec.

SHELF CORRECTION FACTORS

Standards	Shelf No.	Cps.-bkg.	Ave. cps.	Correction Factors
1	4	47.130	43.287	1.00
2		44.071		
3		38.661		
1	5	19.884	18.724	2.31
2		18.726		
3		17.561		
1	6	13.345	12.557	3.44
2		12.14		
3		12.187		
1	7	7.26	6.921	6.25
2		6.825		
3		6.679		
1	8	4.478	4.293	10.08
2		4.253		
3		4.147		
1	9	2.9	2.805	15.43
2		2.77		
3		2.746		
1	10	2.053	1.887	22.94
2		1.849		
3		1.758		

These correction factors should be multiplied with the appropriate shelf counts to correct all values to the 4th shelf of the counting arrangement under the thin mica window of the Geiger Mueller Tube.

THE DETAILS OF SURFACE DENSITIES, WEIGHTS AND
RADIOACTIVITY (IN COUNTS PER SECOND), OF ALL THE SAMPLES

Technique I

Birds	Samples	Surface Density x(mg/cm ²)	Time Intervals	Weights		Ash	Radioactivity	
				Wet	Dry		Am Values*	At Values*
1	Blood	1.87	20 hrs.	0.8612	missed	0.0116	0.123	.1261
2	Blood	0.37	6 hrs.	1.0601	0.1299	0.0038	1.353	1.259
	1/2 cc. Bile	1.28	6 hrs.	0.4678	0.1226	0.0091	1.789	1.8089
	Liver	2.47	6 hrs.	2.3099	0.5652	0.0311	1.996	2.0798
	Spleen	1.11	6 hrs.	0.5091	0.1016	0.0063	1.72	1.7760
	Cecal contents	2.26	6 hrs.	0.6161	0.1554	0.0149	426.386	440.29
	Cecum	1.71	6 hrs.	1.4186	0.2429	0.0147	25.211	26.309
3	Blood	0.70	24 hrs.	1.6010	0.1391	0.0064	0.413	.4184
	0.7 cc. Bile	-	24 hrs.	0.8029	0.1750	0.0151	-	-
	Liver	-	24 hrs.	1.5859	0.4646	0.0227	-	-
	Spleen	-	24 hrs.	0.5901	0.1300	0.0091	-	-
	Cecal contents	5.45	24 hrs.	1.9121	0.5081	0.0589	434.942	484.66
	Cecum	2.00	24 hrs.	1.7917	0.2978	0.015	3.923	4.0952
4	Blood	0.85	8 hrs.	1.1435	0.1766	0.0087	0.212	1.176
	0.8 cc. Bile	0.44	8 hrs.	0.6439	0.1505	0.0048	0.291	.3219
	Liver	4.87	8 hrs.	4.0633	1.0651	0.0614	2.238	2.4706
	Spleen	1.79	8 hrs.	1.1999	0.2734	0.0183	0.209	.2160
	Cecal contents	10.05	8 hrs.	1.799	0.6018	0.0754	322.201	396.76
	Cecum	2.16	8 hrs.	2.0236	0.3529	0.0186	11.181	11.50
5	Blood	0.61	24 hrs.	1.0027	0.1482	0.0062	0	0
	1 cc. Bile	0.55	24 hrs.	0.5929	0.0637	0.0047	0.211	.1956
	Liver	3.51	24 hrs.	3.6484	0.7568	0.0442	1.525	1.6482
	Spleen	3.15	24 hrs.	1.6602	0.3111	0.0252	0.299	.3152
	Cecal contents	21.56	24 hrs.	3.1577	0.7678	0.1423	391.719	605.41
	Cecum	2.10	24 hrs.	2.822	0.4066	0.0265	22.481	23.525

Technique I (Contd.)

Birds	Samples	Surface Density x(mg/cm ²)	Time Intervals	Weights		Radioactivity	
				Wet	Dry	Am Values*	At Values*
6	Cecal contents	1.2	10 min.	0.3068	0.008	0.0059	193.545
	Cecal contents	6.21	1 hr.	0.5153	0.0619	0.0466	197.027
	Cecal contents	1.14	3 hrs.	0.4093	0.0259	0.0056	147.079
	Blood	1.28	3 hrs.	0.8662	0.0904	0.0091	3.342
	Blood	0.81	6 hrs.	0.9247	0.1055	0.0091	1.145
	Entire Liver		6 hrs.	49.1056			
	Slice	5.25	6 hrs.	2.1506	-	0.0299	0.394
	1 cc. Bile	1.91	6 hrs.	0.9673	-	0.0164	1.007
	Spleen	5.01	6 hrs.	4.1281	-	0.0541	0.229
	Cecal contents	10.14	6 hrs.	1.0737	0.284	0.0497	398.426
	Cecum	2.66	6 hrs.	2.2342	0.3395	0.0189	41.977
	Cecal contents	0.02	10 min.	0.4595	0.0051	0.0001	238.269
	Cecal contents	0.54	1 hr.	0.4748	0.031	0.0079	182.106
	Cecal contents	0.86	3 hrs.	0.3397	0.03	0.0083	159.36
7	Blood	1.37	3 hrs.	0.5789	0.0804	0.0085	1.112
	Cecal contents	1.54	19 hrs.	0.2754	0.0251	0.0088	130.211
	Blood	0.96	25 hrs.	0.9146	0.1243	0.0077	0.551
	Blood	1.09	3 hrs.	0.9303	-	0.0087	0.573
	Blood	0.78	24 hrs.	0.7564	-	0.0071	0.215
	Entire Liver			36.9764			
	Slice	6.37	24 hrs.	1.5184	-	0.0223	0.341
	1 cc. Bile	2.23	24 hrs.	0.7708	-	0.0127	0.303
	Spleen	5.37	24 hrs.	2.1163	-	0.0333	0.078
	Cecal contents	3.04	24 hrs.	0.3349	0.0822	0.007	277.654
	Cecum	7.88	24 hrs.	4.4944	-	0.0418	5.442
	Cecal contents	0.60	1 hr.	0.3404	0.0323	0.0058	332.871
	Blood	0.87	3 hrs.	0.9159	-	0.0079	0.289
	Blood	0.87	24 hrs.	0.8636	-	0.0079	0.349
8	Blood	1.32	3 hrs.	0.9759	-	0.0094	0.218
	Blood	0.65	6 hrs.	0.9455	-	0.0073	0.201
	Blood	0.46	10 hrs.	1.086	-	0.0064	0.206
	Blood	0.69	20 hrs.	1.0384	-	0.0055	0.222
	Blood	0.84	24 hrs.	0.9713	-	0.0076	0.282
	Entire Liver						
	Slice	6.37	24 hrs.	1.5184	-	0.0223	0.341
	1 cc. Bile	2.23	24 hrs.	0.7708	-	0.0127	0.303
	Spleen	5.37	24 hrs.	2.1163	-	0.0333	0.078
	Cecal contents	3.04	24 hrs.	0.3349	0.0822	0.007	277.654
	Cecum	7.88	24 hrs.	4.4944	-	0.0418	5.442
	Cecal contents	0.60	1 hr.	0.3404	0.0323	0.0058	332.871
	Blood	0.87	3 hrs.	0.9159	-	0.0079	0.289
	Blood	0.87	24 hrs.	0.8636	-	0.0079	0.349

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Technique I (Contd.)

Birds	Samples	Surface Density x(mg/cm ²)	Time Intervals	Weights		Radioactivity	
				Wet	Dry	Ash	Am Values* At Values*
8	Entire liver			49.5038			
	Slice	5.79	24 hrs.	2.3116	-	0.0359	0.693 .7829
	3/4 cc. Bile	0.77	24 hrs.	0.3094	-	0.0092	0.343 .3359
	Spleen	3.16	24 hrs.	2.1329	-	0.0341	0.371 .3922
	Cecal contents	1.37	24 hrs.	0.4626	0.1192	0.0118	362.89 365.80
	Cecum	2.55	24 hrs.	3.6364	-	0.0275	33.147 34.341
	Cecal contents	0.61	1 hr.	0.5505	0.0232	0.0059	238.992 245.77
	Blood	0.66	24 hrs.	0.5707	-	0.006	0.363 .3468
	Cecal contents	1.45	1 hr.	0.2734	0.0206	0.0071	338.976 338.45
	Blood	0.93	6 hrs.	1.0598	-	0.0074	0.536 .5638
9	Blood	0.86	20 hrs.	0.9163	-	0.0069	0.333 .3239
	Blood	0.60	24 hrs.	1.0238	-	0.0084	0.379 .3834
	Entire liver		24 hrs.	50.0738			
	Slice	2.83	24 hrs.	2.1873	-	0.0272	1.318 1.4148
	Spleen	4.25	24 hrs.	2.1002	-	0.0302	0.491 .5320
	Cecal contents	9.92	24 hrs.	0.8635	0.2780	0.0417	538.71 660.12
	Cecum	2.68	24 hrs.	3.3994	-	0.0289	16.402 17.254
	3/4 cc. Bile	1.66	24 hrs.	0.6879	-	0.0159	0.507 .5136
	Blood	0.66	3 hrs.	0.9132	-	0.006	0.229 .2188
	Blood	0.59	24 hrs.	1.0526	-	0.0067	0.183 .1820
10	Cecal contents	0.10	24 hrs.	0.5143	0.0073	0.0015	5.856 5.8560
	Spleen	3.38	24 hrs.	4.5688	-	0.069	0.265 .2833
	Entire liver		24 hrs.	38.6584			
	Slice	1.39	24 hrs.	1.5285	-	0.02	0.279 .2854
	Cecal contents	11.00	24 hrs.	1.131	0.300	0.046	450.59 567.93
	Cecum	1.32	24 hrs.	2.5284	-	0.021	5.965 6.220
	Cecal contents	1.74	1 hr.	1.0018	0.059	0.0139	526.969 559.57
	Blood	0.81	3 hrs.	1.1057	-	0.0091	0.259 .2668
	Blood	0.64	24 hrs.	1.0651	-	0.0065	0.216 .2331
	Blood	0.46	24 hrs.	0.8816	-	0.007	0.134 .1244
11	Entire liver						
	Slice	1.39	24 hrs.	1.5285	-	0.02	0.279 .2854
	Cecal contents	11.00	24 hrs.	1.131	0.300	0.046	450.59 567.93
	Cecum	1.32	24 hrs.	2.5284	-	0.021	5.965 6.220
	Cecal contents	1.74	1 hr.	1.0018	0.059	0.0139	526.969 559.57
	Blood	0.81	3 hrs.	1.1057	-	0.0091	0.259 .2668
	Blood	0.64	24 hrs.	1.0651	-	0.0065	0.216 .2331
	Blood	0.46	24 hrs.	0.8816	-	0.007	0.134 .1244
	Entire liver						
	Slice	1.39	24 hrs.	1.5285	-	0.02	0.279 .2854

Technique I (Contd.)

Birds	Samples	Surface Density x(mg/cm ²)	Time Intervals	Weights		Ash	Radioactivity	
				Wet	Dry		Am Values*	At Values*
11	3/4 cc. Bile	0.50	24 hrs.	0.7050	-	0.006	0.147	.1483
	Liver	1.74	24 hrs.	0.9738	-	0.015	0.191	.2028
	Spleen	2.10	24 hrs.	1.6133	-	0.025	0.165	.1727
	Cecum	2.17	24 hrs.	3.5624	-	0.033	13.736	14.194
	Cecal contents	3.09	24 hrs.	0.573	0.1287	0.013	292.293	311.44

Technique II

Birds	Trials	Samples	Surface Density x(mg/cm ²)	Time Intervals	Weights		Ash	Radioactivity	
					Wet	Dry		Am Values*	At Values*
1	1	Blood	0.60	1/2 hr.	0.5297	0.0037	0.0037	1.596	1.6143
		Spleen	4.47	1/2 hr.	2.7961	0.0429	0.0429	0.356	.3894
		1/2 cc. Bile	0.43	1/2 hr.	0.3957	0.0063	0.0063	0.412	.4454
		Cecal contents	1.33	1/2 hr.	0.8272	0.0106	0.0106	92.378	97.05
		Cecum	1.94	1/2 hr.	4.8141	0.0381	0.0381	6.503	6.9128
2	1	Liver	3.73	1/2 hr.	2.2639	0.0339	0.0339	0.654	.7136
		Blood	0.91	1/2 hr.	0.7788	0.0073	0.0073	0.216	.2223
		Cecal contents	1.17	1/2 hr.	0.9851	0.0055	0.0055	402.597	439.41
		Blood	0.94	1/2 hr.	0.9255	0.0075	0.0075	0.284	.3020
		Cecal contents	1.12	1/2 hr.	1.7495	0.0126	0.0126	439.578	272.47
3	3	Blood	0.99	1/2 hr.	1.0178	0.0079	0.0079	0.259	.2618
		Cecal contents	2.0	1/2 hr.	1.3735	0.0098	0.0098	402.597	420.27
4	4	Blood	0.74	1/2 hr.	1.0135	0.0067	0.0067	0.302	.3235
		Cecal contents	1.79	1/2 hr.	1.6276	0.0127	0.0127	335.105	346.36

Technique II (Contd.)

Birds	Trials	Samples	Surface Density x(mg/cm ²)	Time Intervals	Weights		Radioactivity	
					Wet	Ash	Am Values#	At Values*
2	5	Blood	0.65	1/2 hr.	1.0111	0.0066	0.326	.3067
		Cecal contents	1.29	1/2 hr.	1.6133	0.0124	403.241	410.9
	6	Blood	0.94	1/2 hr.	1.0651	0.0075	0.573	.6092
		Cecal contents	1.20	1/2 hr.	1.6683	0.0122	521.077	534.23
	7	Blood	0.49	1/2 hr.	0.9788	0.0055	0.341	.3372
		Cecal contents	1.38	1/2 hr.	1.834	0.0132	320.362	325.29
	8	Blood	0.93	1/2 hr.	1.0031	0.0074	0.247	.2598
		Cecal contents	0.93	1/2 hr.	1.3512	0.0066	351.521	369.78
	9	Blood	1.18	1/2 hr.	0.9573	0.0073	0.266	.2682
		Cecal contents	1.05	1/2 hr.	1.29	0.0084	271.585	265.27
	10	Blood	1.44	1/2 hr.	1.005	0.0089	0.248	.2459
		Cecal contents	1.23	1/2 hr.	1.2235	0.0087	258.461	271.6
3	11	Blood	1.58	1/2 hr.	1.0335	0.0098	0.349	.3521
		Cecal contents	1.23	1/2 hr.	1.5137	0.0125	342.127	359.53
	12	Blood	0.97	1/2 hr.	0.9953	0.0088	0.302	.2991
		Cecal contents	1.34	1/2 hr.	1.2646	0.0107	244.342	258.64
	1	Blood	0.85	1 hr.	0.9956	0.0077	0.340	.3269
		Cecal contents	1.06	1 hr.	1.3053	0.0085	341.526	356.76
	2	Blood	0.75	1 hr.	0.9299	0.0076	0.282	.2728
		Cecal contents	0.49	1 hr.	0.9788	0.0053	326.961	323.36
	3	Blood	1.07	1 hr.	0.9742	0.0076	0.227	.2259
		Cecal contents	0.86	1 hr.	1.8508	0.0093	490.88	477.5
	4	Blood	1.11	1 hr.	0.9399	0.0089	0.227	.2344
		Cecal contents	0.96	1 hr.	0.9721	0.0072	203.033	198.98
5		Blood	1.24	1 hr.	1.0176	0.0088	0.213	.2257
		Cecal contents	0.88	1 hr.	0.8224	0.0066	185.694	184.83

Technique III

Birds	Samples	Surface Density x(mg/cm ²)	Time Intervals	Weights		Radioactivity	
				Wet	Ash	Am Values*	At Values*
1	Blood	0.66	0 hr.	0.8514	0.0057	1.357	1.2963
	Entire cecum	8.06	0 hr.	4.2258	0.0645	-	-
2	Blood	0.92	0 hr.	0.8495	0.0065	0.506	.5266
	Entire cecum	8.06	0 hr.	4.6686	0.0645	465.875	550.54
1	Blood	0.87	1/2 hr.	1.1848	0.0089	2.909	2.8626
	Entire cecum	5.30	1/2 hr.	2.5816	0.0376	333.344	373.37
2	Blood	0.67	1/2 hr.	0.9258	0.0068	0.66	.6400
	Entire cecum	5.93	1/2 hr.	2.7087	0.051	272.259	310.34
3	Blood	0.60	1/2 hr.	0.8769	0.0055	0.293	.2964
	Entire cecum	0.80	1/2 hr.	3.5937	0.066	290.295	349.40
4	Blood	0.78	1/2 hr.	0.9884	0.0071	0.299	.2966
	Entire cecum	5.99	1/2 hr.	4.1063	0.0575	297.51	337.69
1	Blood	0.67	1 hr.	0.8794	0.0061	0.379	.3675
	Entire cecum	7.08	1 hr.	4.3321	0.0722	296.1	343.41
2	Blood	0.78	1 hr.	1.0511	0.0071	0.182	.1805
	Entire cecum	3.70	1 hr.	2.7318	0.0355	259.882	281.31
3	Blood	0.66	1 hr.	0.9149	0.006	0.214	.2044
	Entire cecum	7.43	1 hr.	4.4316	0.0758	281.574	330.89
1	Blood	0.76	2 hrs.	1.0019	0.0069	0.413	.3991
	Entire cecum	4.75	2 hrs.	2.2515	0.0337	248.06	270.01
2	Blood	0.76	2 hrs.	0.9417	0.0069	0.236	.2281
	Entire cecum	5.18	2 hrs.	2.3378	0.0471	260.679	290.25
3	Blood	0.75	2 hrs.	1.0433	0.0076	0.174	.1660
	Entire cecum	16.91	2 hrs.	5.5599	0.1353	242.229	343.29
1	Blood	0.72	4 hrs.	0.555	0.0051	0.276	.2876
	Entire cecum	12.4	4 hrs.	3.55	0.0657	264.641	343.05
2	Blood	0.80	4 hrs.	0.7711	0.0082	0.656	.6674
	Entire cecum	8.17	4 hrs.	3.1683	0.0613	241.375	286.08

Technique III (Contd.)

Birds	Samples	Surface Density x(mg/cm ²)	Time Intervals	Weights		Radioactivity	
				Wet	Ash	Am Values*	At Values*
3	Blood	0.69	4 hrs.	0.5486	0.0043	1.092	1.0906
	Entire cecum	7.12	4 hrs.	1.6377	0.0299	272.556	317.89
4	Blood	0.74	4 hrs.	0.8021	0.0067	0.302	.3235
	Entire cecum	7.44	4 hrs.	2.3261	0.0491	286.17	336.74
1	Blood	1.90	6 hrs.	0.9841	0.0093	0.137	.1426
	Entire cecum	21.43	6 hrs.	4.704	0.1222	208.944	322.77
	Liver	6.13	6 hrs.	2.4608	0.0325	0.085	0.974
	1/4 cc. Bile	0.65	6 hrs.	0.2089	0.0052	0.085	.0800
2	Blood	1.46	6 hrs.	0.9575	0.0083	0.137	.1377
	Entire cecum	25.21	6 hrs.	7.01	0.1563	136.437	225.74
	Liver	44.13	6 hrs.	2.4304	0.2339	0.242	.5476
	1/4 cc. Bile	0.70	6 hrs.	0.1896	0.0046	0.222	.2249
3	Blood	0.99	6 hrs.	0.9724	0.0085	0.287	.2901
	Entire cecum	111.65	6 hrs.	5.9219	0.6364	230.906	1134.35
	Liver	6.27	6 hrs.	2.257	0.0307	0.092	.1048
	0.6 cc. Bile	0.22	6 hrs.	0.6188	0.0134	0.066	.7260
1	Blood	1.11	8 hrs.	1.0197	0.0079	0.128	.1322
	Entire cecum	21.94	8 hrs.	4.8652	0.1075	198.54	309.68
	Liver	4.52	8 hrs.	2.0516	0.032	0.139	.1537
	0.6 cc. Bile	1.66	8 hrs.	0.6144	0.0143	3.936	3.985
2	Blood	0.56	8 hrs.	0.9986	0.0057	0.279	.2634
	Entire cecum	10.26	8 hrs.	3.9309	0.1047	289.979	358.95
1	Blood	0.52	16 hrs.	0.8111	0.0059	2.674	2.8065
	Entire cecum	3.82	16 hrs.	2.74	0.0432	266.08	290.22
2	Blood	1.20	16 hrs.	0.8341	0.0085	0.097	0.994
	Entire cecum	6.36	16 hrs.	3.8166	0.0611	231.395	263.55
	Liver	3.21	16 hrs.	2.215	0.0308	0.169	.1815
	1/4 cc. Bile	0.62	16 hrs.	0.1126	0.0033	0.165	.1725
3	Blood	1.03	16 hrs.	0.9136	0.0099	0.110	.1157
	Entire cecum	25.16	16 hrs.	5.1854	0.156	190.137	313.96
	Liver	4.33	16 hrs.	2.8748	0.0416	0.191	.2109
	1/4 cc. Bile	0.32	16 hrs.	0.092	0.0029	0.109	.1172

Technique III (Contd.)

Birds	Samples	Surface Density x(mg/cm ²)	Time Intervals	Weights		Radioactivity	
				Wet	Ash	Am Values*	At Values*
4	Blood	1.02	16 hrs.	0.839	0.0088	0.248	.2582
	Cecum	19.70	16 hrs.	4.1533	0.1123	192.508	287.65
	1/4 cc. Bile	0.80	16 hrs.	0.1601	0.0053	0.119	.1211
	Liver	2.54	16 hrs.	1.8912	0.0259	0.177	.1897
5	Blood	1.38	16 hrs.	0.897	0.0091	0.167	.1696
	Cecum	16.67	16 hrs.	4.3348	0.095	177.249	247.63
	1/2 cc. Bile	1.01	16 hrs.	0.4067	0.0103	0.107	.1103
	Liver	3.23	16 hrs.	2.6505	0.0365	0.168	.1816
1	Blood	0.99	24 hrs.	1.0634	0.0079	0.198	.2001
	Cecum	17.43	24 hrs.	4.5749	0.0854	183.76	263.27
	Liver	13.29	24 hrs.	2.3144	0.0372	0.248	.3269
	Blood	1.13	24 hrs.	1.0327	0.008	0.105	.1104
2	Cecum	29.98	24 hrs.	8.4467	0.2129	167.029	300.46
	Liver	7.85	24 hrs.	3.3587	0.0557	0.115	.1351
	Blood	0.41	24 hrs.	0.9908	0.0072	0.126	.1299
	Cecum	23.24	24 hrs.	3.9676	0.1325	204.129	326.11
3	Liver	7.94	24 hrs.	1.6293	0.0278	0.116	.1380

*Am Values - Observed radioactivity

At Values - True activity without self absorption

At Values of 1 ml. of Standard C⁶⁰ were: 1. 424.84 cps.
2. 297.918 cps.
3. 297.092 cps.
4. 299.878 cps.
5. 372.467 cps.

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